RECENT ADVANCES IN BOTANY

TO

MY FRIEND

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PREFACE

This present addition to the "Recent Advances" Series is intended to cover only a limited aspect of the subject. For example, neither the cytological nor the genetical side of botany has been included to any marked extent, as both these branches will be treated by other authors in future volumes.

The main difficulty that almost invariably confronts an author of a text of this description is what subject-matter shall or shall not be included. It is hoped that a reasonably comprehensive survey has been made of the selected portions of botany treated here and that no really important paper has been missed.

I should like to take this opportunity of thanking numerous friends for their very helpful advice and useful criticisms, more especially do I owe a great debt to Dr. S. Williams, Professor J. Walton, Dr. J. W. Gregor and Dr. Elsie Cadman. I am grateful to Miss Jockel of the Royal Botanic Garden, Edinburgh, for reading the whole of the proofs. I should also like to take this opportunity of expressing my gratitude to Messrs. J. and A. Churchill, who have been ever ready to place their advice and experience at my disposal.

<div align="right">E. BARTON-WRIGHT.</div>

Corstorphine,
    Edinburgh.

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RECENT ADVANCES
IN BOTANY

CHAPTER I

SOME THEORIES REGARDING PLANT STRUCTURE

Size and Form in Plants—Phyllode Theory of Monocotyledonous Leaf—Cotyledon—Carpel Polymorphism.

THE more recent trends in botanical advances have been in the direction of physiology, rather than morphology, and there has been a considerable dearth of morphological contributions within recent years, using the term " morphology " in the restricted sense. There are, however, three morphological studies that require consideration here. The first of these is the so-called " size-factor " in plants, which has been brought into prominence by Professor F. O. Bower, under the title " Size and Form in Plants."

Size and Form in Plants

It has been pointed out that plants, unlike animals, are storers of material, and are not appreciable expenders ; and also, unlike animals, they are capable of indefinite extension in growth with the continual formation of new organs. For example, in a fern sporeling of primitive type there is continual apical growth and a succession of fresh leaves are formed, each larger than the last, and these are borne on a cone of which the tip forms the organic foundation for gradual upward expansion. The result is a body of unstable form from the purely mechanical standpoint, and requires reinforcements such as strut roots ; a similar state of affairs is to be found in many Palms, and also in the screw-pine, *Pandanus*. One line of escape from this condition has been utilised in many groups, namely, cambial activity leading to secondary thickening. An obconical cone, such as has been

described above for a primitive fern or for Palms, also presents a number of difficulties from the physiological aspect, inasmuch as the physiological needs of the upwardly growing shoot have to be met by a number of readjustments in the internal elements.

Mathematically, with increase in size, the strength of a structure increases as the square of the linear dimensions, whereas the mass increases as the cube (Principle of Similarity). In solid objects, with further enlargement of form, provided there be no alteration in shape, the surface increases as the square and the volume as the cube of the linear dimensions. Although this principle of similarity has been extensively applied by zoologists, it has received but scant attention at the hands of botanists. Physiological interchange in plants is conducted through limiting surfaces, both external and internal, and it may be assumed that in cases in which the surface is unbroken, such interchange will be proportional to the area of surface involved. But since bulk increases as the cube and surface only as the square of the linear dimensions, there will be a continuous decrease in the ratio of surface to bulk, leading to physiological inefficiency. Any increased morphological complexity, however, such as fluting or corrugation, would tend to overcome this difficulty and give a wider increase in surface, and thus level up any disproportion between bulk and surface.

The plant body is a structure of diffusive somatic construction, and on this account requires a large surface of interchange between the dead elements (xylem) and the living cells, and the collective surface exposed between tracheal elements and living tissue will therefore be of importance. A case in point can be found among the recently discovered Devonian fossils in the Chert of Rhynie—the Psilophytales. Three of the species, *Rhynia Gwynne-Vaughani*, *Hornea Lignieri* and *Asteroxylon Mackiei*, form a series in which *R. Gwynne-Vaughani* may be taken as the smallest and *A. Mackiei* the largest, whilst *H. Lignieri* holds an intermediate position. In *R. Gwynne-Vaughani* the vascular system consists of a simple solid strand of xylem composed of annular tracheids, with no distinction between proto- and meta-xylem, and the diameter of the xylem is 0·156 mm. In *H. Lignieri*,
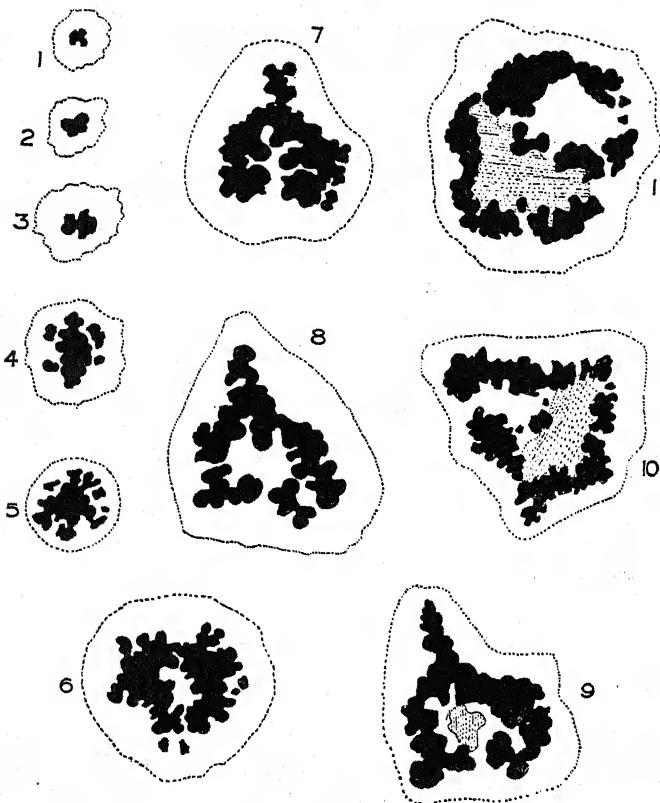
Fig. 1.—*Psilotum triquetrum*. Series of sections taken from base of rhizome into stout aerial stem of same plant. (1–4). Small stele of rhizome. (5). Stele with stellate structure. (6–9). Formation of excentric pith. (10). Large stele from aerial stem. (11). Section taken from thickest part of stem, diameter of stele decreasing. Xylem shown in black, sclerotic tissue stippled, broken boundary line indicates inner limit of · endodermis. (After Wardlaw, *Proc. Roy. Soc. Edin.*)

in which the vascular system is similarly constituted, again with no differentiation between proto- and metaxylem, the diameter of the xylem is somewhat larger, 0·30 mm., whereas in *A. Mackiei*, the largest of these forms (with the exception of the rhizome), the xylem is stellate in structure, and has a diameter of 2·0 mm.

In a still larger species of *Asteroxylon* (*A. elberfeldense*) the diameter of the xylem is 2·8 mm., and there is a further elaboration of the stellate structure together with the formation of a certain amount of pith. Thus in the smaller plants the xylem is approximately cylindrical in shape, whereas in the larger it becomes stellate in construction. The sculpturing of such a solid tract of xylem has the effect of increasing the surface of dead tissue exposed to the living cells surrounding it.

Dr. Claude Wardlaw has recently made some interesting observations on a single rhizome of *Psilotum* from the standpoint of size and form. He followed the enlarging structure from the base upwards, and showed that a simple xylem core exists at the lowest extremity, but with increase in size of the rhizome this simple xylic core increases in transverse section and becomes more elaborate in appearance (Fig. 1), with continual disintegration and the formation of a pith; although, in the later stages, part of the pith tends to become sclerotic in nature. It is necessary here to follow sequences in proportion between the collective

TABLE I

I. Number of Section.	II. Diameter of Stele in mm.	III. Ratio of Bulk to Surface.	IV. Ratio of Bulk to Surface in Equivalent Cylinder.	V. Remarks.
1	0·17	1/9·26	1/6·82	Protostele, xylem solid.
2	0·18	1/7·31	1/4·91	Protostele, xylem solid.
3	0·24	1/5·90	1/4·15	Xylem solid.
4	0·29	1/5·40	1/1·88	Xylem solid, slightly fluted.
5	0·26	1/5·29	1/1·70	Xylem irregularly stellate.
6	0·53	1/3·64	1/1·08	Xylem irregularly stellate.
7	0·56	1/3·12	1/0·97	Irregular excentric pith.
8	0·61	1/3·20	1/0·91	Xylem triangular, pith stellate.
9	0·64	1/3·12	1/0·87	Irregular pith, partly sclerotic.
10	0·75	1/3·00	1/0·81	Xylem interrupted cylinder. Pith sclerotic.
11	0·64	1/3·15	1/0·95	Xylem interrupted cylinder. Pith sclerotic.

surface of dead tracheal tissue actually abutting upon living cells or the *surface of presentation*. These values are given in Table I.

It is evident from column III. of Table I. that the ratio of bulk to surface progressively decreases as the size increases.
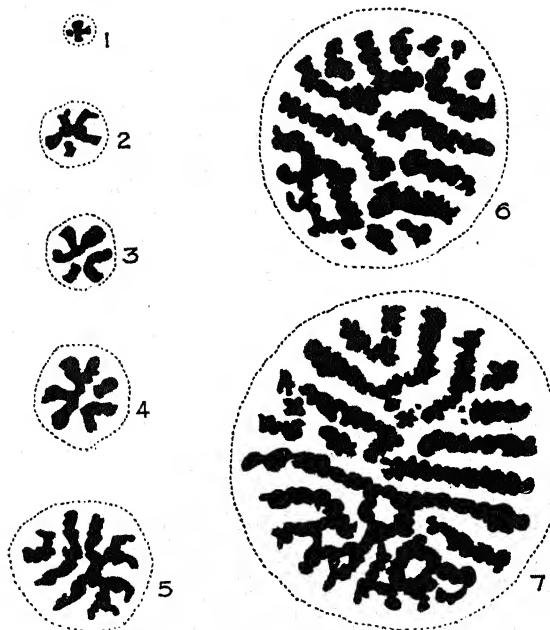
Fig. 2.—*Lycopodium scariosum*, var. *Jussiaei*. (1). Stele of sporeling. (2). Section from a fine distal branch. (3–7). Sections from branches of different thickness. (After Wardlaw, *Proc. Roy. Soc. Edin.*)

Column IV., however, shows like ratios for successive sections, on the assumption that the form of the xylem as a whole is that of a simple cylinder throughout, instead of increasing in complexity of outline. Here proportional diminution in ratio is 1/7th instead of approximately 1/3rd found in column III.

It will be seen that the decrease in the ratio from the simplest stele with a solid core of xylem to the larger and more

disintegrated structure is of such a nature as to maintain a higher proportion of surface to bulk than would be possible on the supposition that the xylem maintained a cylindrical structure throughout. There is also the added physiological advantage that the increasing complexity of form gives over mere enlargement of the original structure, that must be taken into account.

The Lycopodiales, both living and fossil, also furnish interesting examples of size and form. Only a few cases can be given here, and for the arguments advanced in the case of the fossil members of this order, the original memoir should be consulted. In *Lycopodium* itself the stele retains its cylindrical shape, and all increases in size are intra-stelar in nature. Wardlaw has traced the increase in the complexity of the xylem with increase in the size of the stele. In all the species examined there was evidence of branching of the protoxylem groups, and a progressive increase in the phloem and living parenchymatous tissue inwards, so that in transverse section the wood appears to be broken up into separate tracts, although at the same time there is upward and downward continuity between them. In creeping forms, such as *Lycopodium alpinum*, *L. densum*, and *L. volubile*, the xylem shows the appearance of irregular parallel plates. In *L. scariosum* var. *Jussiæi*, Wardlaw made an examination of the condition of the xylem from the state in which it first appeared as a four-rayed star (Fig. 2). The sections were not all taken from the same part of the plant, but from different portions. With increase in size there is sculpturing of the stele, and in transverse section

TABLE II

Name.	Number of Section.	Diameter of Figure in mm.	Number of Protoxylem Groups.	Ratio.
L. scariosum	3	19	6	3·16
,, ,,	4	26	7	3·70
,, ,,	5	38	11	3·45
,, ,,	6	66	19	3·47
,, ,,	7	88	22	4·00

each gives the appearance of a detached plate, but these are connected both in the upward and also in the downward direction. Such a system tends to preserve a high proportion of collective surface to be presented to the living tissue by the bulk of the xylem as a whole (see Table II.).

So far, the examples cited have all been microphyllous forms. The Filicales present a somewhat more difficult problem, inasmuch as they are megaphyllous, and as the leaf increases in size it is subject to the same requirements as the stem that bears it. Since the leaf is of relatively large size, it meets this need by structural changes of the same order as the stem, although they may not be the same in details. For examples furnished by the more primitive Ferns, *e.g.*, Cœnopteridaceæ, Ophioglossaceæ, Marattiaceæ and Osmundaceæ, see Bower, p. 71 (*loc. cit.*). The so-called Leptosporangiate ferns show the highest plasticity of the primary stele, and the continuity of the endodermal sheath with consequent enclosure of the conducting tracts is apparently a matter of importance from the physiological standpoint. The main problem confronting such forms has been to secure an elaboration of stele without disruption of endodermal continuity. The weakest points of such a system, and where possible leakage might occur, would be at the departure of the leaf traces and at the place of conversion from the solenoxylic to the solenostelic condition. In such forms, after a period of
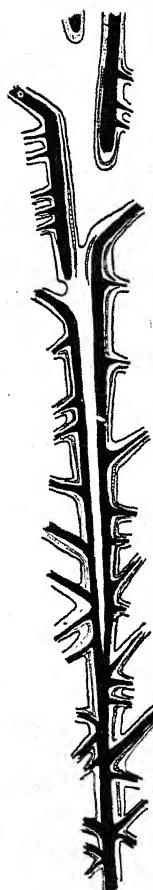

Fɪɢ. 3.—Plan of the stelar construction of a young plant of *Gleichenia pectinata*. It shows in median section the way in which the stele enlarges conically upwards and widens into a solenostele with leafgaps. (After McL. Thompson. From Bower, *Size and Form in Plants*.)

development with increase in amount of pith, internal phloem makes its appearance in the sporeling, and with yet further increase in size the solenostelic condition is established. A series of axillary pockets are now formed, each deeper than the last by involution of the endodermis, and finally the endodermis is continued right across the pith, and an intra-stelar region is cut off both above and below (Fig. 3). Thus the central tract of pith becomes physiologically extra-stelar, although in ontogeny the portion below was intra-stelar. "The sudden structural change is opportunist in character rather than conformable to morphological rule." Such a change allows of a common ventilation-system extending from the cortex through the foliar gaps to the central column, which though geographically intra-stelar is now physiologically extra-stelar. At the same time it doubles the surface of presentation to the ventilated parenchyma. Once solenostely has been established, all further elaborations to dictyostely and polycycly help in the extension of surface. In the leaf meristele, elaboration of structure leading to extension of surface can also be traced. The Osmundaceæ, for example, show the leaf trace at its first inception as an oval mass, which later becomes horseshoe shaped. Further morphological changes in other types of leaf trace leads to the incurving of broken ends.

In its highest condition the xylic tract becomes permeated by living tissue, and in transverse section living and dead cells appear in approximately equal proportion. This "vitalisation" of the dead xylem has only been gradually realised from the primitive protostele, and is brought about by different means, such as medullation, sculpturing and disintegration of the xylem.

In roots, as well as the aerial region of the plant, increase in the ratio of surface to bulk can be traced. Wardlaw has been able to follow this for a Pteridophyte, *Danœa nodosa*, a Dicotyledon, *Gunnera*, and a Monocotyledon, *Colocasia*, and in all three cases found a tendency to preserve a suitable proportion between surface and bulk.

The Principle of Similarity is not confined to the higher plants, but can also be applied to simpler forms, *e.g.* Algæ. In the Green Algæ, for example, the chloroplasts show a large variety of shape

and modification. Bower has calculated the ratio of the diameter
of the chloroplast in the Desmid *Closterium* to the number of
flanges (Table III.), from which it can be seen that their number
shows a relation to size. A fair level of uniformity appears in
the lower terms of the series, but there is a marked rise in the value
of the last member, which is an out-size.

TABLE III

Name.	Mean Diameter in mm. ($\times$ 810)	Number of Flanges.	Ratio of Diameter to Number of Flanges.
Cl. Dianæ . .	10	6	1·6
Cl. juncidium . .	12	7	1·7
Cl. angustatum	20	10	2·0
Cl. striolatum .	23	13	1·8
Cl. Lunula . .	53	15	3·5

In the higher plants, as well as in other families of the Green
Algæ, such as the Siphonaceæ, Ulvaceæ and Characeæ, the chloro-
plasts are small, but nevertheless due proportion is observed
between surface and bulk, since they are discoid in shape, and
on this account are able to maintain a large area of surface for
exposure to light during photosynthesis.

It will be seen from the few examples cited here that the size-
factor is of importance to plants. If the growth of an organism
were to result in mere magnification of the juvenile condition,
it would become both morphologically and physiologically
inefficient. Continued readjustment and amendment of the
original scheme of structure is necessary throughout the growing
stages to meet the fresh demands that continually follow increasing
size, and this can be traced in group after group of vascular plants,
and may even be applied to the lower forms in regard to certain
of their organs. "Morphoplasy," or the study of plasticity of
form of an enlarging organism, as this subject may alternatively
be termed, should give an added impetus to further morphological
studies and investigations.

The Phyllode Theory of the Monocotyledonous Leaf

As far back as 1827 de Candolle suggested that the linear leaf of the Monocotyledons is equivalent to the leaf-base and petiole of a dicotyledonous leaf, the blade of the leaf being unrepresented. This view came to be called the " phyllode theory " of the monocotyledonous leaf. More recently, Mrs. Agnes Arber has brought forward a good deal of anatomical evidence in favour of this original conception.

Dicotyledons and Monocotyledons are, as a rule, though with many exceptions, fairly easily distinguished by their general external appearance. The outward distinction depends partly on the appearance of the leaves and partly on the mode of growth. In Monocotyledons the veins are generally arranged in parallel series, *i.e.*, parallel venation is exhibited, whereas in Dicotyledons the veins form a network. A particularly characteristic feature which makes its appearance in family after family of Monocotyledons is a type of leaf in which a basal leaf-sheath passes distally into a simple limb, which is traversed by a series of parallel veins. The situation is very much more complicated in Dicotyledons, but there is an approach to the monocotyledonous conditions in those Dicotyledons in which so-called "reduced" leaves are present. Such reduced leaves have either no lamina -or the lamina is represented by a midrib. For example, in *Eryngium ebracteatum* and *E. pandanifolium* parallel venation of the leaves is present, but the lamina here is apparently the leaf-base and rachis of a compound pinnate leaf, and the marginal teeth that are found to occur on the lamina represent vestigial pinnules. In monocotyledonous leaves, on the other hand, there is no evidence for the presence of reduced pinnules, or, in fact, that these structures were ever derived from compound leaves.

It is unlikely, on this account, that a leaf of the *Eryngium* type furnishes a suitable comparison with those of Monocotyledons. In any case, such an analogy introduces a number of unnecessary complications. A better comparison is to be found in certain leaf-sheath bases and petiolar phyllodes. Examples of these are to be found in *Petasites vulgaris* (inflorescence foliage), *Hedera*

helix (bud scales) and *Oreomyrrhis linearis* (foliage leaf). In *O. linearis* the leaf consists essentially of an elongated leaf-base, the remaining portion consisting of a minute hooded tip. *Acacia* also represents a Dicotyledon with a reduced type of leaf, and one in which the petiolar phyllode is the most important portion ; a similar state of affairs exists in *Oxalis bupleurifolia* and *Clematis afoliata.*

Arber has pointed out that among Monocotyledons are to be found certain leaves, which from their general appearance and anatomical structure suggest leaf stalks to a remarkable degree, and that their interpretation as petiolar phyllodes " becomes an easy and natural matter." *Triglochin maritimum* and species of *Allium* with their cylindrical leaves are cases in point. For the Iridaceæ, which are possessed of iso-bilateral leaves, a whole series of parallels can be found among the phyllodes of *Acacia*. Thus, in *Acacia uncinella* and *A. neurophylla*, as in the Irids, the median bundle tends to insignificance, while the two principal lateral veins are prominent and form a pseudo-midrib (Fig. 4). The iso-bilateral type of leaf is a feature among Monocotyledons, but is not found among Dicotyledons. For example, they are to be found among the orders Lilifloræ, Farinosæ, Helobieæ and Microspermæ.

Although the phyllode theory finds ready application to radial and ensiform types of leaf, it is somewhat more difficult to apply the theory to ribbon-like leaves with a single series of bundles. But in the Helobieæ, in which ribbon-like leaves are of frequent occurrence, Arber has been able to trace the essential identity of solid petiole-like leaves with leaves of the ribbon-like type. For instance, in the genus *Cymodocea* (Potamogetonaceæ), *C. nodosa* possesses typical ribbon-like leaves, whereas in *C. isœtifolia* a radial structure is present, and *C. manatorum* shows an intermediate condition. Similarly in the Juncaginaceæ, *Triglochin maritimum* has radial leaves, whereas *T. procerum* shows well-developed ribbon limbs.

In *Asparagus* (Liliaceæ) scale leaves are present which exhibit a tail or spine at the base, and these structures are downwardly directed. They are not of the nature of emergences, since they

are the only portions of the leaf which receive a vascular strand directly from the axis, whereas in the upper scale-like region of the leaf, branch bundles enter, and these originate exclusively from the spine bundle (Fig. 5). In this case Arber follows the
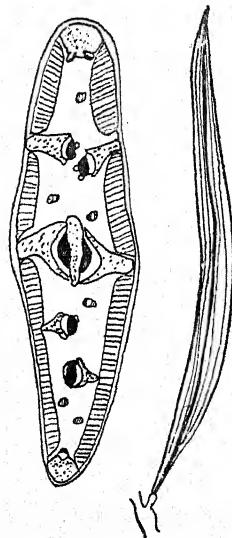
FIG. 4.—*Acacia neurophylla*. (1). A transverse section of phyllode cut not far from the base, showing two upper bundles not yet united. (2). Phyllode. (After Arber, *Mono-cotyledons*.)

FIG. 5.—*Asparagus scaber*. A series of sections through the base of leaf with spine, s. ax = axis, sb = spine bundle, lb = bundles for upper part of leaf. (After Arber, *Monocotyle-dons*.)

interpretation of Buscalioni, namely, that the scale here is a ligular sheath, while the spine is of the nature of a petiolar limb. Although a leaf-base terminating in a simple petiole may be taken as the fundamental type of monocotyledonous leaf type, in which the petiole often forms the conspicuous portion of the leaf, or may on occasion be reduced to a mere trace, or suffer

still further reduction, as, for example, in *Hemerocallis*, in which the petiole is completely lost, there is also another trend in these organs which leads to further elaboration of the petiole. Examples of this latter trend in the evolution of the foliar organs of the Monocotyledons are to be found among the Palmæ and Iridaceæ. In two genera of the Palms, *Cocos* and *Trachycarpus*, if a series of sections be taken through the young plumular leaves (Fig. 6), the sheathing base in each case is succeeded by a short petiolar region. A " pseudo-lamina " is developed by a series of invaginations which penetrate into the tissues between the bundles and result in the formation of a leaf rudiment. This fan-like body ultimately expands, and then, by a process of secondary splitting, the fan or feather-like leaf of the Palm is produced. On the phyllode interpretation this is not a true blade equivalent to the structure found among the Dicotyledons, but is merely the elaboration of the distal region of the petiole. The so-called " ligule " which occurs at the base of the blade of fan-palms " is not a ligule at all, but merely the ventral surface of the proximal part of the petiole, under which the invaginations, which affect the more distal region, have, as it were, burrowed, so as to leave an overarching penthouse of tissue." The dorsal scale which is a conspicuous feature in many Palms can be inter-

FIG. 6.—*Cocos Romanzoffiana*. A series of transverse sections from below upwards through a second foliage leaf of a seedling. (1). Sheath. (2). Petiole. (3–5). Stages in invagination. (After Arber, *Monocotyledons*.)

preted in the same way, while solid tips which are present in certain species (*Phœnix dactylifera*) are to be explained by the dying out of the invaginations before the extreme apex is reached.

Invaginations are also to be found in the Iridaceæ. The *Crocus* leaf, for example, arises from a solid petiole by a single pair of invaginations, which take a somewhat spiral course.

In other monocotyledonous leaves, the petiolar interpretation finds support in the nature of the vascular anatomy of the blade
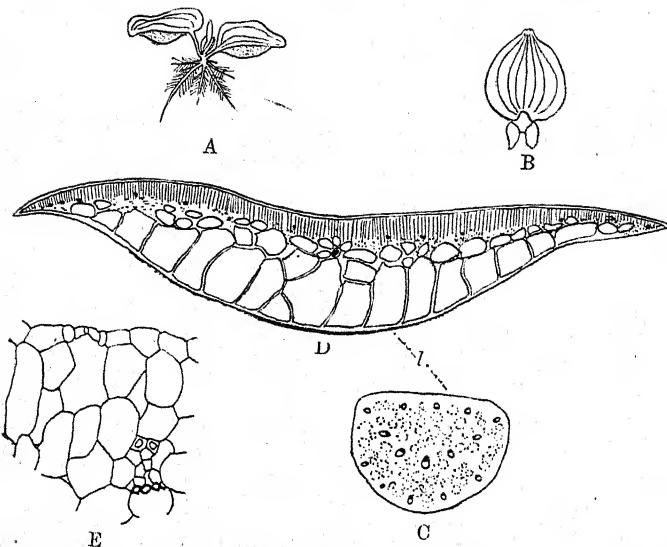
FIG. 7.—*Trianea bogotensis.* (A). Small floating plant. (B). Leaf from adaxial side. (C). Transverse section of petiole of well developed leaf to show radial structure. (D). Transverse section of small leaf limb, palisade tissue shown with straight lines. (E). Small part of the upper surface of a leaf showing a stoma. The xylem is directed downwards, while the phloem lies above. *l* = lacuna. (After Arber, *Monocotyledons*.)

(pseudo-lamina). In the leaf lamina the midrib and principal veins are, as a general rule, supplied by a single series of bundles, in which the xylem is orientated in such a way that it points to the upper or adaxial surface. In the Pontederiaceæ and a number of other families among the Monocotyledons, Arber has discovered the fact that, in addition to the presence of normally orientated bundles, a number of strands are to be found in which the xylem

is directed in a downward direction. In *Trianea bogotensis*, Karst, a small floating plant, which shows a good deal of spongy air tissue, forming a cushion below the leaf blade (Fig. 7), the peculiar orientation of the strands can best be interpreted as due to its origin by flattening and expansion of a petiole of radial type in which the bundles towards the upper surface are inverted compared with those of the lower surface. Arber is in favour of explaining the more complex types of monocotyledonous leaf on the phyllode theory. The theory certainly has a number of attractive advantages, for it very readily explains parallel venation, and even the cordate leaves present in some Monocotyledons can be derived from a parallel-veined organ.

The leaf structure of *Juncus* presents a number of difficulties for the complete acceptance of the phyllode theory. In 1890 Buchenau concluded that the leaf structure in the Juncaceæ can be derived from flat dorsiventral type of leaf which is found in *Luzula*, and also in some species of *Juncus*, and he derived the cylindrical type by supposing that more or less complete suppression of the upper surface had taken place. It was also shown by Goebel, from his study of the junction of the limb and sheath of these leaves, that the radially symmetrical arrangement is derived from a dorsiventral structure by suppression of the adaxial surface. Embryological examination, as well as the study of the early germination stages of the seedling in the Juncaceæ, also show that the first leaf, after the production of the cotyledon, is bifacial.

'Adamson has made a study of twenty-three species of *Juncus*, and favours the older view, that the leaf structure in this family is derived from a dorsiventral form.' Even in the case of *Juncus maritimus*, in which the leaf is externally unifacial, the arrangement of the bundles is dorsiventral. The most complete degree of radial symmetry was found in *J. effusus*, where no structural indication could be found at all of orientation. This species has a large central pith region, and the origin of the radial symmetry of the unifacial leaf from a bifacial one can be easily followed. In the seedling the first leaves are completely bifacial, and the subsequent leaves show a marked decrease in the development

of the adaxial surface, until in the final stage it is completely suppressed, and this suppression is accompanied by a corresponding increase in the extent of the abaxial surface. Whatever may be the mature form of the leaf in *Juncus*, there seems to be no reason to doubt that it has been derived from a parallel-sided bifacial one.

Cotyledon. The nature of the single cotyledon of the Monocotyledons has given rise to almost endless discussion. Whether the seed-leaf of the Monocotyledons is a single or dual foliar organ is the main question.

The seed-leaf of these plants shows a great deal of variation, but in its most characteristic development it consists of a basal sheath, a " ligule," often closed and tubular in form, and a limb, which is regarded by Arber as the morphological equivalent of the petiole of Dicotyledons.

The extensive investigations of Sargant at the beginning of this century showed that a widespread anatomical feature of the seed-leaf of Monocotyledons was the absence of a median bundle, which was replaced by dual strands. Investigation of this anatomical peculiarity led Sargant to the view that the single seed-leaf of this group was in reality a dual organ composed of two fused foliar members. Unfortunately for this view, it has now been shown that dual vascular anatomy is also a feature of many of the seed-leaves of Dicotyledons and even Gymnosperms, and replaces the usual symmetry about a single midrib of the ordinary foliage leaf.

In Dicotyledons a pair of prophylls is usually present on the secondary shoots, whereas among monocotyledonous plants there is only a single prophyll situated between the main axis and a lateral branch. Anatomical evidence points to this single prophyll as being a single foliar organ. If this be the case, it has been suggested by Arber that the seed-leaf of Monocotyledons is also a single foliar member. This suggestion at once rules out Sargant's view of a fused dual organ. It was perhaps unfortunate that in this connection Sargant traced the course of the cotyledonary and hypocotyl bundles from above downwards, and thus fell into the error of treating the root-stele as being composed of

cotyledonary and plumular strands. If, on the other hand, the reverse procedure be adopted, it is found that bilateral symmetry is impressed upon the shoot-stele by the root. The so-called " double bundle " which is of common occurrence in the midribs of dicotyledonous seed-leaves is essentially the continuation upwards of the protoxylem of the root bundle separating two phloem strands. The vascular structure of Monocotyledons shows a greater amount of variation than is to be found among dicotyledonous plants, possibly due to the greater breadth of the sheathing cotyledonary base, which allows the entrance of minor bundles from the root into the cotyledon, and at the same time leads to internodal abbreviation, and brings the primary strands of the primary root into more direct association with plumular traces. It is possible that the exceptions found among Monocotyledons to the rule that the cotyledonary strand is a continuation of the root bundle are due to the fact that many of the seedling roots of this class are either diarch or tetrach. In certain instances the cotyledon is supplied with the whole vascular system of the root, and gives the impression of dual symmetry of the organ. Thus in one type duality is due to the fact that the cotyledon receives a pair of bundles or a single dual bundle which represents the upward prolongation of one root-pole, whereas, in the second type, the cotyledon receives two bundles, each of which represents half of the entire root-stele. Sargant, in her investigations, compared the seedling of *Anemarrhena*, in which two bundles pass up into a single cotyledon, with *Anemarrhena*-like Dicotyledons (*e.g.*, *Opuntia*), in which one of these bundles supplies each cotyledon, as an indication that the cotyledon of *Anemarrhena* represents the condition of two dicotyledonous seed-leaves which have fused together. If, however, the course of the bundles be traced in the upward direction, it can at once be seen that the cotyledon of *Anemarrhena* is a fully developed organ at a stage in which the plumule is in too embryonic a condition to receive strands from the primary root, so that all the bundles comprising the root-stele pass upwards into the sheathing cotyledon because there is nowhere else for them to go.

The suggestion has also been put forward that the single seed-leaf of Monocotyledons has been brought about by division of labour, and a number of investigations have been made with this view in mind. There is, however, a good deal to be said for Arber's standpoint, that too much reverence has been paid in the past toward the "cotyledon." When all is said and done, cotyledons are, after all, the first leaves of the plant, and not organs *sui generis*, and if, as is generally assumed, the Mono-cotyledons form a natural group of plants, why should there ever have been a second cotyledon? It is a quite reasonable hypothesis to assume that their internal metabolism was of such a nature as to lead to the formation of a single seed-leaf, rather than two, and there is also the further fact that in this group, leaf-bases show a marked tendency to ensheath completely the axis and thereby make the production of a second leaf impossible.

Carpel Polymorphism

From the appearance of certain exceptional types of fruit among different genera of the Cruciferæ, Miss Saunders has advanced the suggestion, which now goes under the general title of *Carpel Polymorphism*, that the older view of one uniform type or pattern of carpel through the whole range of the Angiospermæ, is no longer a tenable hypothesis. The spasmodic occurrence of types which are often called "monstrosi-ties," among the fruit of Cruciferous plants, as well as in other natural families, has led her to advance the view that there has been reduction and consolidation of the parts of the gynœcium of Angiosperms, and that this process of consolidation has brought about the formation of certain definite types of carpel forms through the redistribution of carpellary functions.

On the older orthodox tenets of botanical teaching, the mono-carpellary ovary is composed of a single specialised leaf, which has been folded lengthwise and inwards, with the result that the uniting edges cohere, and in this manner have given rise to marginal placentæ. In the case of ovaries composed of more than one member, each carpel differentiates in contact with its neighbours, and the contiguous sides of adjacent carpels have not

been delimited, the one from the other. In these circumstances the syncarpous gynœcium formed in this manner may exhibit either axile, parietal, or, on occasion, free-central and even (but rarely) superficial placentation.

On the polymorphic view of carpel formation, several types of carpel are considered to have arisen in the course of evolution, accompanied by a redistribution and separation of carpellary functions. Saunders recognises three principal types of carpel : (*a*) *Valve-type* or *Hollow Carpel*. This is thought to be the most primitive form, and retains as its typical feature a more or less leaf-like form. As a general rule a midrib is present externally, and reticulate venation is also present. If a style be developed, it is formed by an upward prolongation of the midrib, and bears a single stigma. (*b*) *Solid Carpel*. This is considered to have been evolved from the valve-type described above. In its most highly developed form it not only completely separates the radial surfaces of carpels on either side, but at the same time it may also show a further development by projecting into the ovary cavity, thereby bringing about so-called " false partition " (*cf.* Cruciferæ). In its most reduced form it is composed merely of a fibro-vascular cord with a few lateral veins connecting it with its neighbours. Occasionally the solid carpel is associated with true valve-type of carpels of the same whorl (the majority of Cruciferæ), or even with carpels of a different whorl (four-valved Cruciferæ and Primulaceæ), and sometimes with the third type of carpel described by Saunders, the so-called *pseudo-valve* type (see below), as in *Fritillaria Meleagris* and the Orchidaceæ. (*c*) *Pseudo-valve* or *Semi-Solid Carpel*. This particular type of carpel combines the features of both the valve- and solid-type mentioned above. In its external features it has the contour of the valve-type, but the placentæ are displaced from the contact edges of the carpels and take up a position on either side of the centre line. The vascular strands are also differently arranged from the valve-type, and show a double central strand (midrib) from which connections run direct to the funicles, whilst other branches supply the ovary wall. The presence of this type of venation enables the semi-solid carpel to

split at maturity down the centre between the two bundles of the midrib (*cf.* Papilionatæ). Like the solid-type, the pseudo-valve carpel is also found in association with the valve-type and solid-type. When the gynœcium is entirely composed of the pseudo-valve type the carpels may either be all fertile (*Begonia*) or only half of them may bear ovules, and the remaining portion be sterile (*e.g.*, *Fritillaria Imperialis*, L. Liliaceæ and *Hæmatoxylon campechianum*, L. Cæsalpinieæ).

Saunders visualises the phylogenetic history of the gynœcium as follows : In primitive forms, in which only valve carpels are to be found, the several members would at first be visible as a number of protuberances. The vascular bundles which are eventually destined to form midribs would become differentiated and curve outwards and upwards. Concurrently with this development there would be " ballooning " in an outward direction of the ground tissue on either side of a midrib, and this would lead to the formation of a loculus. With further expansion of the now hollow carpels, the gynœcium would show the apocarpous or syncarpous condition, depending on whether the carpels be sufficiently distant from one another to enable each to accomplish this development separately from its neighbours, or be differentiated at points so near together that the development of the sides of adjacent carpels as separate entities could not take place. When the carpels are not sufficiently distant from one another, or are so near that the development of the sides of adjacent carpels is prevented as discrete entities, radial plates of tissue would make their appearance, each composed of a common side wall of two contiguous carpels with the consequent development of a plurilocular ovary. The first disposition would result in giving rise to marginal placentation, the second to axile placentation. The condition known as parietal placentation could be brought about in a number of different ways. For example, as the circumference of the ovary enlarged, these radial plates might thin out and leave the centre as incomplete partitions, or the carpels may be conceived as abutting on each other by their edges, which just meet and cohere. Alternatively, they may be of such a width as not only to meet and cohere, but also to project

inwards. The development of the many-carpelled plurilocular ovary is thought to be brought about by the outward expansion of the centre of each carpel, bringing the sides with it at the same time. In cases in which a second whorl of carpels is retained, the two whorls may arise at a level so near together, that with further development they give the appearance of constituting a single whorl. • One very definite trend in evolution is reduction in the number of parts of an organ, as well as differentiation in form. Such reduction, it may be supposed, first led, as far as the gynœcium of the flower is concerned, to the suppression of the second inner whorl of carpels, and this can be seen in the act of taking place in *Triglochin palustre*, **L.** (Juncaginaceæ) and *Zanthorhiza apiifolia*, L'Herit (Ranunculaceæ), in which the inner whorl is becoming smaller and sterile. Once the stage of reduction of the inner whorl is reached, in which it is largely suppressed, or even wholly repressed, the outer whorl may also begin to lose individual members. • Reduction, however, can also be brought about by other means, namely, by diminution in bulk, and polymorphism will again ensue. Thus in the valve carpel the lamina might disappear, and the midrib alone would be left, or even on occasion merely its vascular cord. If this consolidation took place in the outer whorl, it might conceivably act as a predisposing cause of the assumption of semi-solid or pseudo-valve form of carpel in the inner whorl, which perhaps, already on its way to assuming the solid-form, would now have its evolutionary trend changed in a new direction. There is also the possibility that the primitive valve carpel can be converted into the pseudo-valve form independently of the steps described above, since in some species (*e.g.*, *Fritillaria Imperialis*) both whorls are composed of semi-solid or pseudo-valve carpels.

In a further class of cases the members of the outer whorl can be conceived as failing to outcurve and expand to produce the loculus. Here their vascular cords will remain in the central position, while those of the inner whorl would be able to expand and produce typical valves (*Geranium*, *Erodium*, *Pelargonium*). The reverse situation is met with in the Cruciferæ. According to Saunders, the immediate ancestors of the present living forms of

this family may be taken as possessing a gynœcium composed of an outer whorl of four orthogonal valve carpels and an inner whorl of four diagonal solid members.

With rise in polymorphism, the stigma-bearing and ovule-bearing functions often came to be performed by one kind of carpel only. But although the distribution of stigmas and placentæ has generally become a constant feature for a species, and even for a whole genus, in neither case can it be said to be the outcome of any fixed interrelationship between one type of carpel and another. On this account the two functions must be investigated in each case.

It will be impossible here to give a full account of Saunders' ... investigations on carpel polymorphism, and only a few selected examples ... be chosen from the very large mass of data presented in her ... papers. The originals should be consulted in ... order to ob... complete record of this very painstaking work. The examples ... here are taken from among both Dicotyledons ... as well as Mono...edons in an endeavour to show the full implications of the the...

Dicotyledons. Cruciferæ ... G4. On the polymorphic view the family becomes G4–8–∞ ...valve and ...solid in silicula.

In the majority of genera in wh... fruit is a siliqua, two median solid and two lateral valve ... present. Reduction in this case has apparently reach... the ... point of stability. The solid carpels are invariably fertile ... the majority of cases ...bear the (commissural) stigmas; the valve carpels are nearly always sterile and destitute of stigmas. In genera in which more than four carpels occur ... number is often indicated by various external features, e.g. wing-like outgrowths and prominent ribs. Biscutella, ...carpels sterile, valve carpels fertile and contributing ...ation of style and stigma, and Matthiola, stigmas centred ... valves, represent the reverse condition to that described ...which one valve is ... Biscutella fruits make their appearance ...

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completely absent. Nevertheless, even in these circumstances, replum and style are similar in form to normal fruits, a further proof that they cannot be formed from the margins of two lateral carpels, since only one is present.

In those Cruciferæ in which the fruit is a silicula, the dehiscent section is usually subdivided into the two groups : *latiseptæ*, with a broad and *angustiseptæ* with a narrow dissepiment. In two common Cruciferous plants with this type of fruit, *Lunaria annua* (Honesty) and *Capsella Bursa-pastoris* (Shepherd's Purse), the venation of the fruit shows that the median carpels in both cases are pseudo-valve in nature (*cf.* siliqua-type of fruit) with wide lateral expansions, while the lateral carpel pair have undergone a corresponding reduction. In *L. annua* the fruit is extended in the median plane, and consequently the replum is broad from back to front, and the sides of the fruit are also broad. At the time of maturity the sides become detached from the replum-like true valves in the siliqua, but,

FIG. 8.—(1). *Capsella Bursa-pastoris.* Fruit showing venation systems arising from two solid carpels (right and left) and from semi-solid carpel (centre). (2). *Lunaria annua.* One of the fruit valves, showing main veins which are derived from three different carpels. (After Saunders, *Anns. Bot.*)

unlike this structure, they do not each represent an individual lateral carpel, which is here represented as a small undefined region of the large oval valve face. The midrib can be seen as a small vein which runs up the centre of this face for a sh... distance before breaking into reticulations which connect the ultimate ramifications of the median semi-solid (Fig. 8). These latter members are folded in such that the two halves of the lamina face each other, and midrib run along the edges of the fruits and eventually ... style, which terminates in a bifid stigma ... *Capsella Bursa-pastoris* is much the same ... fruit is flattened from back to front ...

L. annua

angles to the plane of flattening in *Lunaria*, and the sh... the carpel pairs is reversed. The two different venation sy... of *Capsella* fruit are very clear (Fi... one springing from the midribs of... semi-solid pair of carpels with a num... of strong lateral ribs, the other from... midrib of the reduced valve memb... which merely give rise to a few latera... veins.

Evolution of the gynœcium has apparently moved along two different paths in the Cruciferæ as the result of carpel polymorphism. When the median carpels took on the solid-type characteristics, the characteristic shape of the fruit became a siliqua, and when they became semi-solid in nature the result was a silicula.

The Berberidaceæ also furnish an example of carpel polymorphism. On the usual view G = 1 here, but on the polymorphic view G becomes 2. one carpel, composed of a small valve and the other large and semi-solid. *Epimedium* is a good example. The fruit when ripe splits down the two sides along the border at which the two systems of venation, one arising from the midrib of a sterile valve carpel, and the other from the central strand of a fertile semi-solid carpel, meet but do not unite (Fig. 9). When dehiscence occurs the sterile valve carpel, which is smaller than the other, becomes detached, leaving behind the now opened-up semi-solid fertile structure bearing the style and centrally placed... bearing the style and centrally placed... two rows of ovules.

...ceæ (including the Funariaceæ) G is described
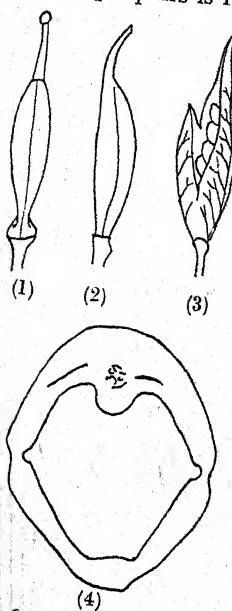
FIG. 9.—*Epimedium alpinum.* (1). Young fruit showing valve view. (2). The same in side view. (3). Ripe fruit already dehisced, showing two systems of venation, one arising from the midrib of the sterile valve and the other from the midrib of the fertile semi-solid carpel. (4). Ovary in transverse section showing vascular bundles the two midribs and thin places in the wall, and left) where the carpels unite and asunder. (After ... *Bot.*

comple... ... from the viewpoint of carpel polymorphism G becomes
replu... rarely 3; carpels almost invariably alternative valve and
proof rarely all valve or all solid. The minimum number of
carpels is apparently three (*Platystigma*, Benth.) and the gynœcium

I... eemingly composed entirely of valve carpels; the styles and
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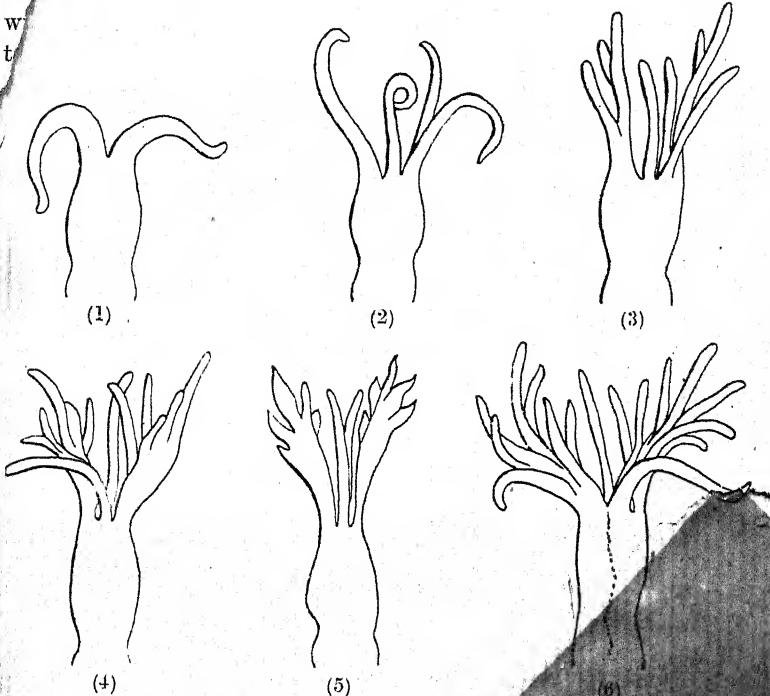
FIG. 10.—*Eschscholtzia californica.* Semi-diagrammatic representa-
tion, showing the order of disappearance of the styles. The
earliest stage is shown in (6) and the final stage in (1). (After
Saunders, *Anns. Bot.*)

stigmas stand over the valves. In the closely related g...
Eschscholtzia, Dendromecon and *Hunnemannia*, a "comr...
valve is found. This structure simulates a single ty...
carpel, but is composed of numerous sterile soli...
members, which become detached together as a s...
Eschscholtzia, in which the styles are distinct, a...

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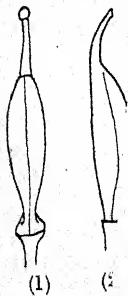
of constant occurrence. In the ancestral form it is possible that all twenty carpels must have normally produced styles. Now, the usual number during the early part of the season is four, and this later becomes reduced to two. If, however, the earliest flowers be examined, it is not an unusual feature to find among them as many as eight, twelve and even sixteen style-like structures, and
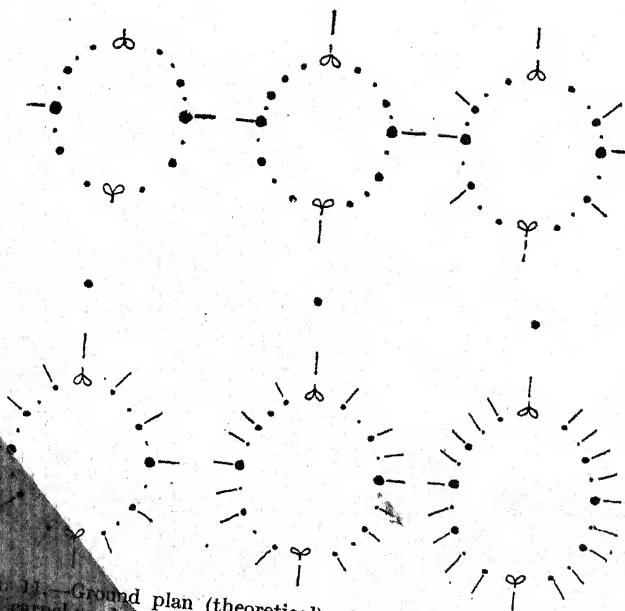

Fig. 11.—Ground plan (theoretical) of an ovary in which every carpel produces a style. (After Saunders, *Anns. Bot.*)

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Carpels

their order of disappearance is very regular, and takes the following course: the styles are first lost from some of the eight-valve carpels and then from the whole number of valve carpels, leaving only twelve (i.e., those belonging to the ten sterile and two fertile carpels, Fig. 10), then from the outer solid members of each lateral group of five, styles are also lost, leaving eight (Fig. 10). Following upon this, the outer members of the two lateral groups three also lose their styles, with the result that the typical

four-styled condition with two lateral and two median members
is obtained (Fig. 10). Finally, the replum-styles also disappear
and only the central style of each compound valve remains. (For
a diagrammatic representation of this process see Fig. 11.)

Monocotyledons. The Liliaceæ supply a series of convenient
examples for a discussion of the theory of carpel polymorphism
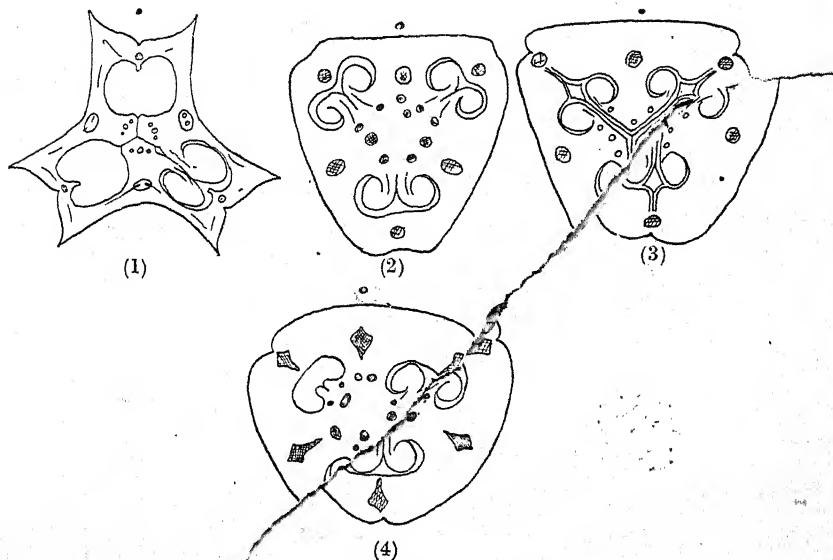
FIG. 12.—Ovaries of various members of the Liliaceæ in transverse
section. (1) *Fritillaria Imperialis*. (2). *F. Meleagris*. (3).
Tulipa Gesneriana. (4). *Lilium Martagon*. (After Saunders,
Anns. Bot.)

among monocotyledonous plants. G is (3) here on, becomes 3 + 3 from the polymorph
view, but *Tulipa* and *Lilium* furnish a series of
Fritillaria, for discussion. In *Fritillaria Imperialis*
fruit, which shows in cross-
six-winged, one being situated in the
stress six cords, six systems
midribs of six semi-solid

possess no placentæ, and are situated opposite the loculi, and their midrib bundles are continued upwards into the styles. Alternating with these are the layer of fertile carpels, which are semi-solid in nature. It is only at the extreme base of the ovary, and extending upward for a short distance at the point at which the vascular cords of the sterile carpels run outwards and loculi make their appearance, that the septa form a continuous mass of tissue at the centre (Fig. 12, 1). Throughout the rest of the length of the ovary, the central ends, though in contact, are delimited and the whichfaces are indicated by the double layer of epidermal cells point of tan be seen in transverse section, extending from the mid-line (Fig. 12, e section to the three loculi as a three-rayed bounding-veins to right 1). Each of the six midribs gives rise to parallel lateral each carpel with and left, and these extend to the line of junction of the sterile carpels its neighbour (Fig. 12, 3). On their inner surfaces the tissue over the mhow a slight protuberence into the loculus of tissue are seen to have drib. At the flowering stage these arcs of that which lines the rest e an epidermis of different character to istically large and exhibit arf the loculus. The cells are character-the remaining epidermal cells deeply staining outer wall, whereas tangentially and present an even sound the loculus are flattened manner as the remaining tissue. Irface, and stain in the same sharp, and this is claimed to be the preis change is particularly epidermis of the sterile carpel ends and theise point at which the commences. In *F. Meleagris* the sterile membat of its fertile fellow have undergone a further stage of consolidaters of the gynœcium *Imperialis* and are all of the solid type (Fig. 12, 2 ion to that of *F. Tulipa Gesneriana* may be considered as a third). series. G is again 3 + 3, and the relative pos ird member of ta are very similar to *F. Meleagris*. The deitions of loculi rfaces, however, is not accomplished as elimitation of in, the convex arc, with its deep early as in jecting from the inner surface of ly staining pposite the angle, which is chara the ovary here. On the other hand, at the po istic is made, a few epidermal ha ut

(1) (2) (3) (iii) (4)

SAMMETTA

(5) (6) (7) (8)

Fig. 1.— ... types of styles, stigmas and fruits in the Liliaceæ
... carpel polymorphism. (1). *Lachnocaulon anceps*.
... orphic styles and stigmas, the commissural stig...
... *Papalanthus brachypus*. The same as (1) but
... commissural stigmas. (3). *Fritillaria Imperialis*
... dehiscence, showing venation system arising from
... on solid carpels, the splitting of the midrib
... the tearing in halves of the finger-like process
... he loculus. (4). *Tulipa Gesneriana*. Young fl...
... of stigmas. (5). Transverse section throu...
... of *T. Gesneriana*. (6). The same, hi...
... red, showing a large number of scattere...
... dots) derived from semi-solid carpels...
... by short lines) at the end of each stig...
... carpels. (7). Ripe fruit of *T. Gesneri*...
... owing central venation system arising
... of the semi-solid carpels (fruit-valve)
... smaller system arising from the so...
... turned so as to bring one of the fruit...
... owing splitting of solid carpel. (Af...

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sionary, and the question
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Saunders mainly relies on an...
the theory of carpel polymorphis...
out, there is no clear comparative ev...
of a functional solid carpel.

According to Saunders, the ancestor ...
a four-valvular carpellary ovary with par...
present carpel was obtained through one p...
but losing ovules, whereas the other becar...
but retained fertility with ovules near the m...
these an ingrowth now took place, the two fu...
septum (replum). It is this structure which ha...
such difficulties in the interpretation of the Cruci...
On the orthodox view, the replum is formed by ...
the two placentæ, which subsequently fuse. W...
on this matter, the older view may be quite as pr...
explanation put forward by Saunders.

To take another example, the Leguminosæ, on th...
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used to demonstrate this view is *Hæmatoxylon*. W...
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the Leguminosæ. The pod dehisces down the middle
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Saunders explains this by supposing that the gynœciun...
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down the lines of juncture. Only one of these carpel...
to be fertile, bearing ovules on its midrib, and th...
the majority of the Leguminosæ is regarded as b...
a fertile semi-solid and a sterile solid carpel. It ...
Hæmatoxylon, which can scarcely be consider...
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grow out into the loculus from either side of the cleavage line. These occur at this particular place because here is the meeting point of two actual edges of the carpels, and, further, the presence of the loculus, a cavity, allows of hair formation. The sterile carpels in the Tulip have suffered even greater consolidation than that of *Fritillaria Meleagris*, and although they have retained their midrib, the semi-solid fertile carpels have engulfed them in front and behind.

The final example given here of carpel polymorphism among Monocotyledons is *Lilium*. In *L. Martagon* the general plan is similar to *Tulipa*, but the delimitation of the centre surfaces of the semi-solid carpels is not apparent. The placentæ form a continuous tissue throughout the greater part, if not the whole of the ovary. All trace is also lost of the process whereby the solid carpels have become hidden from sight on the side bordering on the loculus, and in the epidermal layer there is no sign either of a clear line of cleavage or the formation of hairs. The semi-solid carpels have not only closed over and buried the last remnant of the midrib of the reduced carpels, but such complete coalescence has taken place that no sign of fusion remains on this face.

In all three genera it is the outer whorl which has undergone consolidation and reduction in bulk. This can be seen from its position opposite the sepals, and also from the fact that in sections cut immediately below the loculi the midribs of the sterile carpels lie farther back from the centre than the vascular strands of the fertile members of the gynœcium.

Saunders' views on carpel polymorphism have not met with complete acceptance. Parkin regards the implications of the theory as being of too sweeping a nature, and that it can only be reasonably applied to the fruits of the Cruciferæ. As Parkin has pointed out, the theory was originally started from a study of the abnormal fruits of the Stock, and that as double number of carpels were found in the abnormal forms was a reversionary feature, and therefore that the typical gynœcium in the Cruciferæ is composed of four and not two carpels. There is always the danger of the temptation of using such abnormal developments to support theories, and such arguments are often held not to

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sionary, and the question
between the two ?

Saunders mainly relies on an...
the theory of carpel polymorphis...
out, there is no clear comparative ev...
of a functional solid carpel.

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but losing ovules, whereas the other becar...
but retained fertility with ovules near the m...
these an ingrowth now took place, the two fu...
septum (replum). It is this structure which ha...
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On the orthodox view, the replum is formed by...
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down the lines of juncture. Only one of these carpel...
to be fertile, bearing ovules on its midrib, and th...
the majority of the Leguminosæ is regarded as b...
a fertile semi-solid and a sterile solid carpel. It...
Hæmatoxylon, which can scarcely be consider...
genus in this family, should differ in carpellar...
the rest of the family.

Again, Saunders gives on...
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base. A few years later (1926) Ashby devised a method of making transfers which was a great improvement on Walton's original technique. Walton (1928a) has now incorporated part of Ashby's technique into his Canada balsam method. The revised procedure is described below:—

(i.) **The Preparation of a Canada Balsam Transfer.** (a) A piece of rock with the fossil upon it is trimmed to a convenient shape by cutting or chipping. If the surface be *very* uneven it must, if possible, be levelled.

(b) This part of the process is necessary only when it is not certain whether the preparation is to be kept in a form suitable for examination by reflected light or by transmitted light. The surface of the rock with the specimen upon it is covered with a solution of cellulose acetate in amyl acetate (a few drops of the solution are allowed to spread over the surface). The surface is then allowed to dry. There is now a film of cellulose acetate adhering to the surface. The solution must not be too concentrated or the film will not adhere with sufficient firmness.

(c) A glass slide is taken and a sufficient amount of balsam is placed upon it (the balsam should be in the form of a viscous solution in xylol). The slide is then placed on a hot copper plate and heated until most of the xylol is driven off. The specimen, which has been warmed to the same temperature, is then placed face downwards on the hot balsam and moved about to expel air-bubbles, and the preparation is allowed to cool.

(d) If much rock projects from the back of the specimen this should be removed by careful grinding.

(e) The preparation is washed in water and dried.

(f) The surface of the rock at the back of the specimen is covered with water, and the whole preparation is then dipped in melted paraffin wax. Several coats of wax should be applied on top of the other until the whole slide and specimen are covered completely. The wax covering the back of the rock is cut away with a knife. The preparation is now placed into a bath of hydrofluoric acid and kept overnight therein. By the morning the rock

converted into mud by the solution of the silica it contains and the fossil is left adhering to the balsam surface.

(*h*) The wax is now cut away from the slide and any excess of balsam scraped off. The specimen is gently washed in water to remove acid and allowed to dry, after which it may be examined under a binocular microscope.

If it be found that the important features of the fossil can be seen by reflected light and that the surface can be examined in relief, the transfer can be protected by constructing a small glass case over it by means of a cover-slip supported on strips of glass or other suitable material of the correct thickness. If, however, it be discovered that the specimen is translucent and that it can be examined by transmitted light, the characters shown in surface relief being relatively unimportant, then it can be removed from the slide by dissolving away the Canada balsam in xylol, and the specimen remains attached to the film of cellulose, which can be mounted in a xylol solution of balsam under a cover-slip, in the same way as in the method shortly to be described below.

When the balsam transfer method is applied for the isolation of large fragments of plant remains and for the preparation of cuticles, it is unnecessary to employ cellulose acetate, and the fragments are finally obtained by simply dissolving away the balsam in xylol.

(ii.) **Ashby's Cellulose-Film Transfer Method.** It is a great advantage when the fossil material exposed on a rock consists of a very thin film of organic material, or is even composed of small fragments and separate particles (*e.g.*, spores), to examine the material by transmitted light and to have it in the form of a flat film. Ashby's cellulose-film transfer method allows of this to be done.

The surface to be transferred is treated with a solution of cellulose acetate in amyl alcohol. Other similar solutions can be used, *e.g.*, the trade preparation " necol," or a solution of celloidin.

(*b*) The surface is allowed to dry thoroughly and, if necessary, the above treatment is repeated in order to obtain a sufficiently strong film.

2—2

(*c*) Superfluous rock is ground away, in order to lessen the mass to be dissolved.

(*d*) The specimen is placed in hydrofluoric acid in a wax vessel until the cellulose film is freed and clear of mineral matter.

(*e*) The transfer is washed thoroughly in water.

(*f*) Dehydrated in 95 per cent. alcohol (absolute alcohol must not be used).

(*g*) Cleared in terpinol or oil of bergamot (clove oil must not be used).

(*h*) Mounted in Canada balsam, slight pressure being applied with a clip if necessary.

The preparation must now be placed in a warm place until the balsam is properly hardened off.

Peel-Methods of Preparing Sections of Fossil Plants. Walton, as well as Barnes and Duerden, have recently described methods of preparing sections from petrified material by using cellulose esters and gelatin. In his first description, Walton employed various cellulose esters to the petrified surface, but he has now abandoned these for gelatin. The Barnes and Duerden method also depends on the use of cellulose esters, and will be described first, as it differs from Walton's in several respects, inasmuch as the cellulose is dissolved away at the end of the process and the finished preparation is left as a balsam mount.

The silicified surface to be etched is first smoothed by rubbing it down with a thin paste of water and carborundum powder on a sheet of thick glass. For the preliminary smoothing a coarse powder is employed (No. 90), and this is followed by a finer grade of powder (No. 220), and finally by ordinary knife powder. The surface is washed with water and then etched with 2 per cent. hydrochloric acid for ten minutes in the case of coal ball material, while siliceous material gives good results after six to eight minutes in strong commercial hydrofluoric acid. After etching, the blocks are washed in water for a few minutes, and should acid still be present, they may be further washed in weak sodium carbonate solution or exposed to gaseous ammonia. They are finally dried by gentle heating.

The dry blocks are now supported with the etched face upwards

and covered with sufficient absolute alcohol to form a layer over the flat surface, and then covered with a solution of celluloid in equal parts of absolute alcohol and ethyl ether. Enough of the solution is poured over the surface to form a layer of about 2 mm. thick. It is necessary at this stage that all bubbles should be removed, either by exposing the film to the fumes of ethyl ether or by wetting the skin with absolute alcohol and then allowing the fumes of ether to spread over the surface.

The film of celluloid dries in about two to three hours to a tough film, and is removed from the surface of the rock by means of a sharp knife. The transfer is now well washed in water, dried with blotting-paper, smeared with Meyer's egg albumen, placed on a well-heated slide and quickly pressed down. When it is clear that the transfer is firmly attached to the slide, the whole is placed in a mixture of equal parts of alcohol and ethyl ether. The celluloid swells and dissolves, and when solution is complete, the preparation is finished as a balsam mount in the usual way.

This method suffers from the defect that the area of surface to be covered with the celluloid solution cannot, from the nature of the process, be very large, which is a material drawback in certain cases. A gelatin peel-method recently described by Walton (1928b, 1930) overcomes this difficulty.

After the surface of the material has been cleaned and etched in the manner described above and washed, just before the surface is completely dry, a hot solution of jelly containing a certain quantity of glycerine and formalin is poured over it. The actual quantities and proportions that are necessary have to be determined by experiment. To cover a surface of 1 sq. decimetre it is necessary to use about 2 gms. of fairly pure gelatin, 50 c.c. of water and 0·5 c.c. glycerine, and 0·5 c.c. formalin (40 per cent.). The surface must be surrounded before the etching process with a rim of plasticene or some other substance, and should be levelled with a spirit level. The water and glycerine are mixed, heated, and the jelly stirred until dissolved. The heating is continued until the mixture is at a temperature of 60° C. to 80° C. The formalin is now stirred in quickly and the solution poured immediately over the surface of the petrifaction and the whole is allowed

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to dry. When the jelly has become dry it may be peeled off. The peeling process is sometimes rather difficult, as the film of gelatin is more brittle than a similar cellulose film. The gelatin peels may be cleared in xylol and mounted in Canada balsam.

The preparations obtained in this way are remarkably clear and show no detectable flaw. Walton has been able to prepare large sections of about 20 sq. decimetres in area by this method.

Aspects of the Palæozoic Flora

Devonian. Since the publication of the now classical memoirs of Kidston and Lang on the plant remains found in the Chert of Rhynie in Aberdeenshire (Scotland), which have been fully described by Scott (*Studies in Fossil Botany*, Vol. I.), a number of descriptions have appeared from time to time on plant remains of Devonian age.

In 1925 Lang described a plant, *Zosterophyllum myretonianum*, from the Carmyllie beds of the Lower Old Red Sandstone of Scotland, which are of Lower Devonian age. This was a relatively small plant, consisting of a branch-system of leafless axes, which were traversed by a central vascular strand composed of annular tracheids. There is a good deal of evidence to show that these erect, branched axes sprang from a rhizomatous region. The most important feature of the discovery, however, was the fact that the terminal regions of the aerial axes bore more or less closely associated, stalked, reniform appendages, often with a marginal rim to the appendage. The arrangement is a loose spike, and the general appearance of the appendages is very suggestive of their being reniform sporangia, although the presence of spores was not detected. On this ground alone it is a pure assumption to suggest that these are sporangia, but the more recent discoveries of plant remains in Australian beds of Lower Devonian age practically puts the matter beyond dispute (see below).

Lang and Cookson have described remains from the Walhalla series of Victoria, Australia. These rocks are described as of Upper Silurian age by the Geological Survey of Victoria, but

Lang and Cookson prefer a more conservative estimate, and consider them to belong to the Lower Devonian.

Four important types were found : (1) *Hostimella*, sp., which is composed of branched axes, and is recognised as of the same general type as those found in Europe ; (2) vascular strands showing tracheidal structure ; (3) a fructification, *Zosterophyllum australianum* ; and (4) a sporangium-like fossil, *Sporogonites Chapmani*.

The chief remains discovered in these beds was *Hostimella.* This consists of smooth-branched dichotomised axes, and is very similar to correspondingly named remains from the Early Devonian of Europe.

The axes are smooth and show dichotomous or lateral branching, suggesting the production of a sympodium from dichotomy. Such remains are of frequent occurrence in Devonian rocks from different regions and may conveniently be kept under the name *Hostimella.* No exactly similar specimens have been described from the Upper Devonian, and as far as our present knowledge goes they are characteristic of Middle Devonian formations. These remains, taken alone, would indicate a Middle Devonian age for the Walhalla rocks, but the associated fossils must also be taken into consideration in this connection.

With regard to the tracheidal elements, it is possible that these may belong to *Hostimella,* but actual proof is wanting. Their presence, at any rate, indicates that vascular plants were in existence on this horizon.

Three specimens of *Zosterophyllum australianum,* nov. sp., were discovered. These have proved to be a new fructification. The fructifications consisted of a naked unbranched stalk with a number of spirally arranged appendages which have proved to be sporangia. The sporangia were large and dehisced by a split running along the extended distal edge, and correspond to those found in *Z. myretonianum* from the Lower Old Red Sandstone of Scotland. The Australian plant can be placed as a new species of this genus. The clear comparison of this plant with that found in the Lower Devonian of Scotland is in favour of placing the Australian beds as being of the same age.

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The other fructification found in these rocks, *Sporogonites Chapmani*, nov. sp., is closely comparable with the fructification described by Halle under the name *Sporogonites exuberans* from the Lower Devonian of Röragen, Norway. Unfortunately, *S. Chapmani* is only an impression, whereas Halle's plant was a petrifaction, so that the internal structure of the former is quite unknown.

The total length of the specimen was about 2·5 cm., and consisted of a slender unbranched stalk about 0·75 mm. in width. This widens gradually to form a basal region to the terminal capsule-like structure, which is about 5 mm. in length.

Another specimen that was also discovered, *S. Chapmani* f. *minor*, though similar to *S. Chapmani*, was much smaller.

The discovery of closely similar plants in the small flora of Australia to those found at Röragen in Europe, is in itself a very striking feature. That *S. exuberans* is associated with such characteristic Lower Devonian remains as *Arthrostigma gracile* and *Psilophyton princeps* is again strong evidence that the Australian beds are of Lower Devonian age.

A further important discovery that falls to be recorded here is a petrifaction from the Burdekin beds of Queensland, which are of Middle Devonian age, to which has been given the name *Schizopodium Davidi*, nov. sp.

This petrifaction, which has been described by Harris in a recent memoir, shows a stele composed of stellate-shaped xylem, surrounded by a narrow zone of phloem. The protoxylem, which is variously exarch or mesarch, shows scalariform pits, while the metaxylem possesses multiseriate pitting. The stele is surrounded by a three-zoned cortex. There is a broad inner cortex of thin-walled parenchyma, a middle zone of thick-walled parenchymatous cells, and this is followed by an outer cortex of collenchymatous cells. The stem appears to have been destitute of leaves, and no trace is discoverable of anything approaching a leaf-trace. On the other hand, only the cortical tissues of the base are accurately known, and the cortex of the smallest axes is missing. No trace of assimilating tissue could be discovered, which suggests that the stem was not assimilating.

The general appearance of the internal structure of the stele is strongly suggestive of *Asteroxylon* (Fig. 14), and provisionally *Schizopodium* may be placed in the Asteroxyleæ. The two genera have the common feature of the Psilophytales, namely, slender axes, and there is also the stellate xylem with protoxylem near the ends of the rays. The pitting of the tracheids is very similar to that of *A. elberfeldense*. The stele, however, differs from *Asteroxylon* in this respect : in *Asteroxylon* it is round, whereas in *Schizopodium* it follows the contour of the wood. This fact alone justifies a generic separation, although it is not sufficient to place it in a different family.

Other Devonian discoveries in recent years are *Asteroxylon elberfeldense* (Kräusel and Weyland), a larger plant than *W. Mackiei*. *A. elberfeldense* differs from *A. Mackiei* in the fact that parenchyma-ous cells are present in the wood. It was altogether a more robust plant than the other form, and Kräusel and Weyland's reconstruction is given in Fig. 15. It is considered by these authors to

FIG. 14.—*Schizopodium Davidi*. Transverse section of a large axis showing the outer cortex which has collapsed round the xylem. (After Harris, *Phil. Trans. Roy. Soc. (Lond.)*.)

at aquatic. The rhizome grew under water and sent up tip-shoots, which in their distal portions were naked and frequently coiled at their tips.

Kräusel and Weyland have also made an addition to the Cladoxyleæ. They have obtained a plant from the Middle Devonian of Germany, *Cladoxylon scoparium*, Kr. and W. The branch is usually stated to have a polystelic structure. On
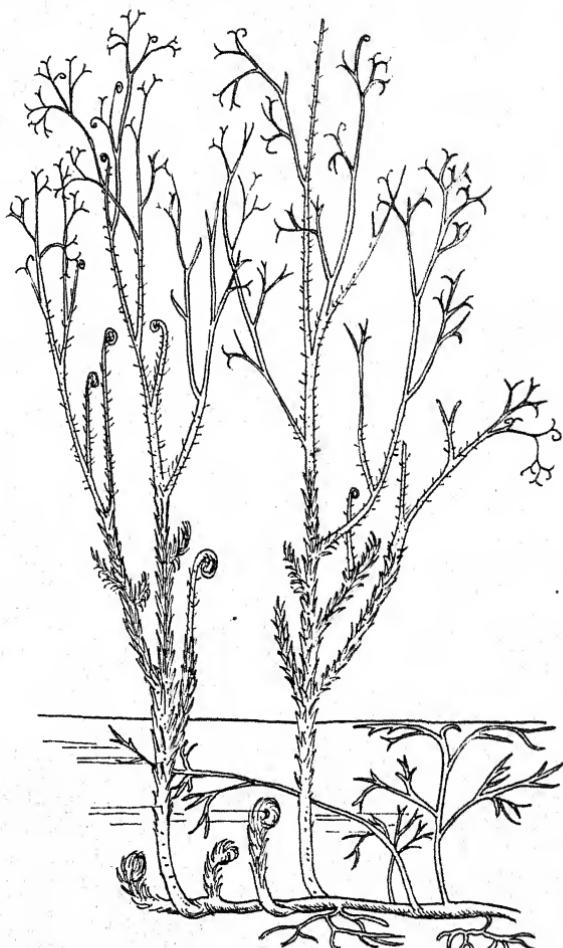
Fig. 15.—Reconstruction of *Asteroxylon elberfeldense*. (After Kräusel and Weyland, *Abhand. Senck. naturforsch. Ges.*)

the other hand, as Harris has pointed out, it is quite poss[...] *Cladoxylon* can be regarded as a somewhat dissected [...] monostele, similar in essentials to a dissected *Schizopodi[...]* Kräusel regards the Cladoxyleæ as a family of mor[...] plants belonging to the Psilophytalean complex. The [...]

ferous members of this group are so different from contemporaneous plants that Hirmer has elevated them to ordinal rank.

Two important investigations on gymnospermous plants from the Upper Devonian have recently been published. Goldring has examined the petrified remains of a forest in Upper Devonian strata at Gilboa in the Catskills (Schoharie County, U.S.A.), which is the oldest forest at present known. In 1869 specimens from this district were sent to Dawson for examination, who identified them as belonging to the tree fern, *Psaronius*, and distinguished two species, *P. textilis* and *P. erianus*. More careful examination has shown that these remains are pteridospermous, and Goldring has renamed the two species *Eospermatopteris textilis* and *E. erianus*.

The stumps were bulbous at the base, possibly because the plants were growing under swampy conditions, and tapered upwards into the trunk. Judging from the stumps and portions of stumps, *Eospermatopteris* must have been a fair-sized tree from 30 to 40 ft. in height. Only the outer cortex has been at all well preserved, and is found to be somewhat similar to *Lyginopteris* and *Heterangium*. The cortex consists of interlacing strands of sclerenchymatous tissue forming a more or less parallel network. In transverse section, unlike the Carboniferous forms, the sclerenchyma is distinguished as dots or short irregular lines. The zone of the outer cortex varies from one to several inches in breadth, depending upon the size of the stumps. The roots are long and strap-shaped. The fronds are compound, tripinnate and from 6 to 9 ft. in length. The pinnules were bilobed and the lobes slightly recurved. The seeds bear a strong resemblance to *Lyginopteris oldhamia*. They were borne in pairs at the ends of forked branchlets, and in all probability near the tips of the frond. The seed was broadly oval, varying in size from 5·3 mm. by 2·5 mm. to 6·4 mm. by 3·4 mm., and were enclosed in an outer husk or cupule. Sporangia-bearing organs (male organs ?) were also discovered. They were modified pinnules, rounded-oval or saucer-shaped to funnel-shaped, and borne on branching pedicels. Goldring's restoration is shown in Fig. 16.

The suggestion is put forward that these plants grew on a low

swampy shore. The evidence for this view is twofold; in the first place, the roots were not heavy and were inadequate for the support of a tree 30 or more feet in height, and secondly, the
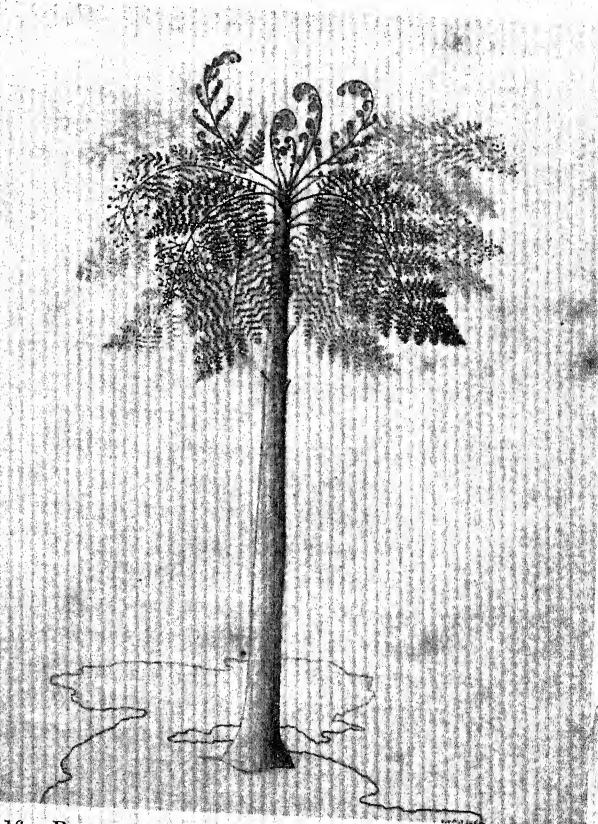

FIG. 16.—Restoration of *Eospermatopteris*, showing the bulbous base, gradually tapering trunk and the crown of large fronds bearing at the tip, in some cases, the seeds and spore-bearing organs. (After Goldring, *Sci. Monthly.*)

presence of a bulbous base, which was very possibly buried in the swampy mud and helped in support.

Plant remains in the Genesee shale and Portage beds of North America, which are of Upper Devonian age, have been critically

examined by Arnold. The remains belong to the genus *Callixylon*, which was established in 1911 by Zalessky. In all, some twenty-four specimens were discovered, and four new species described : *Callixylon Zalesskyi*, *C. mentethense*, *C. erianum*, and *C. bristolense*.

The primary wood in these species is mesarch, and in contact with the secondary wood, although in some cases a few bars of parenchyma may intervene. The actual number of bundles was found to vary between nine and twenty-four. These anastomose freely, forming a network surrounding the pith. The protoxylem elements are composed of annular tracheids; immediately follow-ing these are reticulate, rather than scalariform structures, and then come two or three tracheids with continuous pitting, and finally tracheids with grouped pits. The metaxylem between the pith and protoxylem is mostly composed of spiral tracheæ. The transition zone from annular to pitted condition is relatively brief.

The leaf trace originates from a circum-medullary bundle, and is initiated by a slight increase in size of the bundle. At a slightly higher level, the protoxylem divides. Still further up, the bundle itself divides and the space between the two becomes filled with parenchyma. As the trace works outwards and away from the reparatory strand, the parenchymatously-filled space corres-pondingly lengthens, and later secondary wood commences to close in just outside the reparatory strand, thereby separating the parenchyma-filled space.

In tangential sections of some of the specimens, areas of " convoluted " tracheids were discovered. These depart from the usual elongated form and assume the most extraordinary shapes.

The anatomy of the root shows a group of large round tracheids in the centre of the stele with exarch protoxylem and in contact with the secondary wood.

With regard to the habit of these plants, *Callixylon erianum* possessed stems 2 ins. in diameter; the majority of the other specimens were smaller. The size and length of some of the preserved remains, together with well-marked gymnospermous wood, suggests a tree-habit, in which branches were few, but long

and straight. On the other hand, a slight but natural flattening found among some of the specimens gives support to the view that the plant may have been a vine. In *C. Zalesskyi*, four traces were discovered to arise in a length of about 2 cm., and nine could be seen in various stages of departure. Since no two were vertically aligned, the leaves were spirally arranged, and the phyllotaxy was probably high. The size of the traces and the general distribution suggests that the leaves were larger than those of modern Conifers, but probably smaller than the very large leaves of the Cordaites.

Callixylon had a remarkably wide distribution in the Upper Devonian. Its remains have been found in both Europe and North America. This fact, together with its features of high specialisation, probable arboreal habit, and abundance of remains, suggests that it was one of the commonest plant types of Upper Devonian strata. It is not an unusual feature to find predomin-ance of only a single plant type in a formation, but the situation here is somewhat out of the ordinary, since the distribution is over such a wide area. It is difficult to explain this fact except on the assumption that it was the only plant of the time with resistant tissues.

With regard to the relationships of *Callixylon* and other phylogenetic problems, from its more general characteristics, Arnold is inclined to the view that it is more closely related to the Calamopityeæ and the Pityeæ than the Cordaiteæ. The comparative abundance of *Callixylon* and the rarity of Calamo-pityeæ and Pityeæ gives considerable colour to the suggestion that phylogenetically this genus is of importance. Although it retains a primitive feature, in the possession of mesarch primary wood, the plant itself cannot be considered primitive, " but rather it shows evidence of being the termination of a long—or if short —intensive evolutionary line."

The leaf-gap condition in *Callixylon* is similar to that of *Calamopitys americana* and *Archæopitys Eastmanii* of the Lower Carboniferous. In both *Archæopitys* and *Calamopitys* the reparatory strand is continuous and unbroken at the departure of the trace. There is also a resemblance to *Calamopitys fascicu-*

laris in the fact that the primary wood bundles enlarge slightly and become centrally mesarch before the giving off of a leaf-trace.

As to the relationship with *Pitys* and *Archæopitys*, medullary bundles occur in both these genera, but the situation is very different from the few and scattered medullary tracheids of *Callixylon*. There is also a difference in the number of primary wood bundles; there are some thirty to forty in *Pitys antiqua*. These differences suggest a relationship towards the Calamopityeæ, and thus towards the Pteridospermæ rather than towards the Cordaitales.

There is also some slight resemblance to the little-known *Palæopitys Milleri*, but the evidence is not very clear.

The whole of the evidence at present points to the view that *Callixylon* was a highly organised plant which flourished during the Upper Devonian to much the same extent as the Cordaitales did during the Carboniferous. It reached its climax at the end of the Devonian and disappeared. It was probably not on the direct line of descent of the Cordaites, and possibly sprang from the common stock which gave rise to the Pteridosperms on the one hand and the Cordaites on the other.

Certain points with regard to plant distribution in the Lower Devonian can conveniently be considered here. Lang and Cookson's discoveries in the Walhalla beds of Australia, when taken together, show a close comparison with remains found in Europe. For example, *Sporogonites exuberans* from the Lower Devonian of Norway is so close to the Australian specimen (*S. Chapmani*) that the plants can be placed in the same genus; and, again, *Zosterophyllum australianum* and *Z. myretonianum* (from the Old Red Sandstone of Scotland) show remarkable similarities in their morphological features. The European beds are clearly of Lower Devonian age, while the Australian have been described as belonging to the Upper Silurian. The similarity of the fossil forms, however, lends more support towards Lang and Cookson's view that the Australian beds belong more properly to the Lower Devonian. If this be the case, it is of great botanical interest to meet a similar assemblage of plants as far apart as
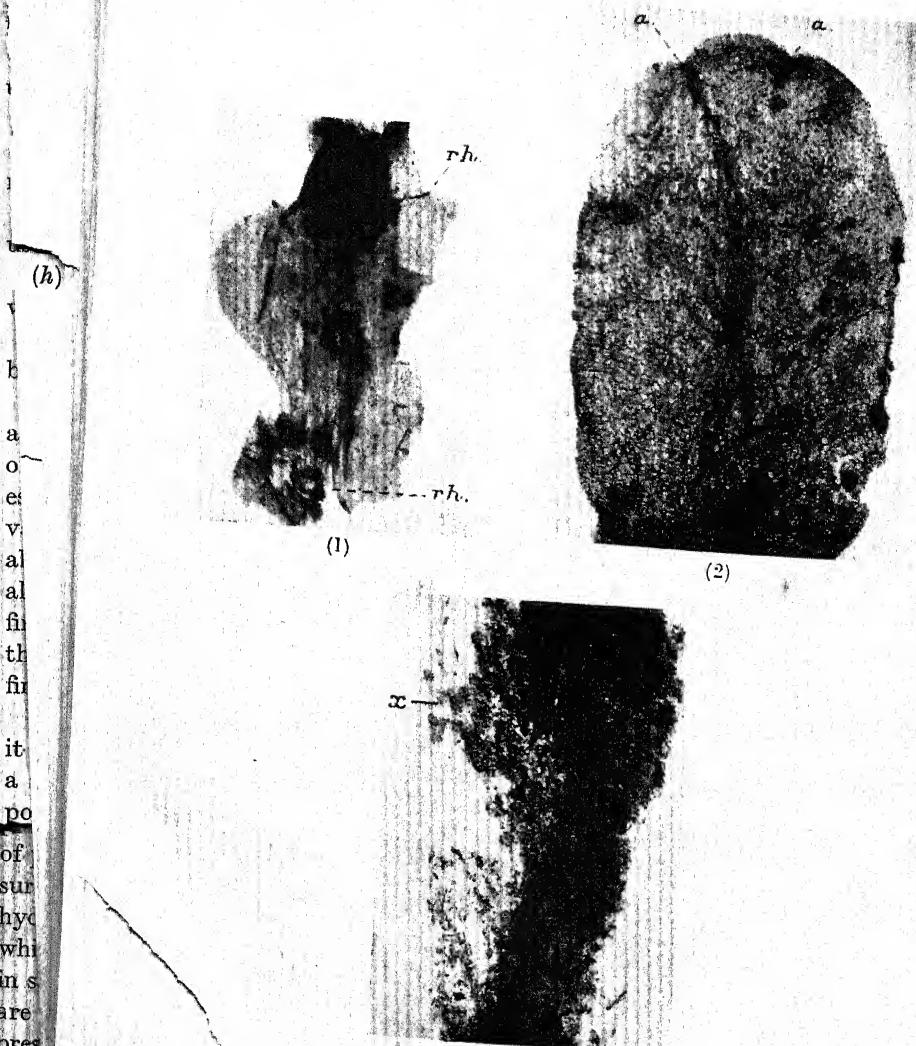
FIG. 18.—(1). *Hepaticites lobatus*, showing part of vegetative shoot ; *rh.*, rhizoids. (2) *H. Willsi*, a shoot about to form a dichotomy ; *a.*, apical notch of branch. (3). Problematical fossil, (?) Sporogonium. Portion of specimen, showing a dense mass of spores and the membrane, *x.*, surrounding them. (After Walton, *Anns. Bot.*)

axial region which are darker than the remainder ; this is apparently due to the presence of fungi. In some of the specimens of this species, rhizoids were found attached in groups. These are somewhat sparingly distributed, and a few at least show a slightly expanded, rounded tip.

Hepaticites Willsi shows a distinct notch in the terminal portion of the thallus, indicating that the growing-point was carried forward in a sunken position. The thallus is a flat ribbon-shaped structure, with its apical cell retained at the base of a notch caused by the initially greater rate of growth of the marginal cells formed at the growing point. Dichotomy is almost certainly indicated by the division into two of the apical cell (Fig. 18, 2).

The fourth species, *Hepaticites Langi,* is made up of small dichotomously branched shoots (Fig. 17, 2) composed of small thin-walled parenchyma. As far as can be seen, the shoot is constructed possibly by the division of a single apical cell sunk by the initially greater growth of the marginal cells in an apical notch and is a smaller plant than *H. Willsi.*

The last species described by Walton, *Hepaticites Metzgerioides,* was discovered in small fragments. The plant, again, is thalloid, with a clearly defined midrib (Fig. 17, 3), and with a wing or lamina one cell in thickness. The midrib is composed of cells elongated in a direction parallel to the axis of the thallus. There is a certain amount of evidence to show that the thallus branched dichotomously. A particular kind of fungal mycelium was found in this specimen. There is also evidence that rhizoids were present.

There can be no doubt that these various specimens described by Walton are true Bryophytes, and that they probably belong to the Anacrogynæ. *Hepaticites Kidstoni* is very similar in the arrangement of its leaves or lobes to the wing in *Treubia insignis,* and somewhat resembles a small edition of that species. If a shoot of *Treubia* be imagined in which each small dorsal lobe has been displaced to a position slightly lower down the axis until it is opposite the middle of its main lateral lobe, and if the decurrent ridges were also suppressed, then a plant very like *H. Kidstoni* would be obtained. *H. lobatus* takes an intermediate position

between *H. Kidstoni* and the simpler thalloid Bryophytes, and in the arrangement of its lobes it resembles the less vigorous shoots of some of the Fossombroniæ. In *H. Willsi* and *H. Langi* the thallus is parenchymatous, with no midrib, and the organisation of the thallus is very like that of *Aneura*. *H. Metzgerioides* shows a very clear distinction between midrib and wing, and is closely comparable with the living *Metzgeria*. In the latter the conducting cells are larger than the other genera that have been mentioned here, and a similar arrangement exists in the fossil *H. Metzgerioides*.

As far back as 1907, Campbell concluded, from geographical distribution and other reasons, that the Liverworts were an ancient group. This view is certainly borne out by the discoveries enumerated here.

A problematical fossil sporogonium (?) has also been described by Walton. The specimen measures some 9 mm. in length and between 0·12 and 0·17 mm. in width. Spores, all of the same kind, were found to be present in the upper region of this structure. It is unlikely that this specimen is an accidental assemblage of spores. There is no indication that the spores were of fungal origin, and, moreover, they are all of the same kind; and lastly, there are no known Palæozoic spores which are so narrow in relation to their length (Fig. 18, 3).

The specimen is, however, such a mere fragment that its exact position is very difficult to define. At present fossil evidence of the Musci is very meagre, and reliable data of their presence in the early rocks are extremely scarce.

Glossopteris and Gigantopteris Flora. The close of the Palæozoic and the inauguration of the Mesozoic Era marks a vast geological revolution. The end of the Carboniferous and the beginning of the Permian was a time when the humid swamps with their lush flora were transferred into hilly regions and relatively dry land, or into desert wastes in which estuaries and fresh-water lakes were converted into inland seas, such as the Caspian Sea. It was also a time of considerable volcanic activity, and the character of the vegetation showed a particularly marked reaction to this change in climatic conditions. The arid

appearance at this time in Australia, and conditions were favour-
able for the accumulation of ice and snow. This Ice Age was of
particularly wide extension, for boulder clays have been found
in every part of Gondwanaland. In fact, Gondwanaland may
be compared with Alaska and Greenland of to-day, *i.e.*, ice sheets
and glaciers with a border of Arctic vegetation.

One of the commonest plant remains found in Gondwanaland
is called *Glossopteris*. Its leaves were long (12 ins.), and it
was in all probability a Pteridosperm. From the abundance of
its leaves and stems (*Vertebraria*), the Gondwanaland flora is
often spoken of as the *Glossopteris* flora. Another common fossil
of this region is *Gangamopteris*. Its leaves were larger than
Glossopteris, and may be distinguished by feeble development
of the midrib. *Schizoneura* (Articulatæ) and *Neuropteridium*,
represented by simple pinnate fronds, are also features of the
Gondwanaland flora. These four genera are not found north
of the Tethys Sea, nor are they represented in the Carboniferous
and Lower Permian. Associated with this *Glossopteris* flora is a
plant which is possibly related to the Cordaites, *Nœggerathiopsis*,
and a few species of *Sigillaria* and *Lepidodendron*. Along with
these, and making a point of contact with the northern flora,
were *Psaronius*, *Sphenophyllum*, and *Psygmophyllum*, which pos-
sessed lobed leaves very similar to those of *Gingko biloba*. This
southern flora was mainly wanting in the genera and species found
in the two northern continents, and was relatively a meagre one.

There was also apparently a migration northwards of this
Glossopteris flora, either *via* land bridges or islands, across the
Tethys Sea, since *Glossopteris* leaves have been found in the
Upper Permian of North Russia and from several localities in
Siberia. This northern *Glossopteris* flora is called the " Kusnezk
flora," from a locality in Siberia, and has been traced as far east
as Vladivostok. It includes with *Glossopteris*, such genera as
Neuropteris, *Callipteridium* and *Lepidodendron*. Thus *Glossopteris*,
which originated, as far as we know, in the Southern Hemisphere,
gradually spread across the Equator, and has recently been
recognised as far north as in the Rhætic flora of East Greenland.
Schizoneura has also been recognised in the Early Triassic of

Western Europe. These facts illustrate that the *Glossop...* flora penetrated into the Early Mesozoic.

Halle has recently published an important memoir on coal-bearing beds of north-western Shansi, central Shansi western Tai-yuan-fu in China, special attention being paid the distribution of the flora. The plant-bearing beds in th... regions are of Permian and supposedly Lower Mesozoic age, a... may be divided into three series, which in ascending order are

(1) *Yuehmenkou Series.* Black argillaceous shales, dark gr... calcareous shales, coal seams and light-coloured quartz-sandston... interbedded with dark marine limestone and calcareous shales.

(2) *Shihhotse Series.* Light-coloured fresh-water and de... deposits, without marine intercalations and almost without co... seams, but very rich in fossil plants. The whole of the beds this series may be regarded as being of Upper Permian age.

(3) *Shihchienfeng Series.* The beds in this series were form... under more or less arid conditions, and are composed of red-brow... or chocolate-coloured claystones, sandy clays, marls and red-brow... sandstones.

The chemical composition of these beds has been investigat... by Norin. The Shihhotse series is of special interest in th... connection because of their abundant plant remains, and becaus... of the peculiar lithological character of the sediments. As far a... can be said, the whole of the beds in this series were laid down... a continuous sedimentary sequence, and, proceeding upwards, at... certain stratigraphical *niveau*, a change in the chemical characte... of the sediments is met with and heralds a change in the nature... of the flora, and a new flora, the *Gigantopteris* flora, makes its... appearance. Chemical investigation indicates that the sediments... of the Upper Shihhotse series were derived from rocks which had... previously been subjected to intense laterite weathering, and... that climatic conditions were favourable for this process to take... place during the deposition of the beds. The climate at that... time was tropical, with an annual alternation of wet and dry... periods. In the Lower Shihhotse series, laterites are missing,... and when these beds were being laid down the climate was of a... more temperate character. Both the sediments of the Shihhotse...

and Yuehmenkou series have the common feature of delta and fresh-water formations, deposited in lakes with drainage to the sea. At the time, however, when the beds between the Shihhotse and Shihchienfeng series were being laid down, a marked geological change took place, whereby these regions, which had previously possessed outward egress to the sea, lost this feature and became embedded in the marginal zone of a desert region. The sandstone division of the Shihchienfeng series, with its masses of diagonally bedded sand, represents the end phase of the sanding-up of this undrained basin, and in this series no plant remains have been found.

The geological age of these various beds is difficult to assess. Halle considered that in the Yuehmenkou series, from the nature of the plant remains and comparison with similar remains in Europe and elsewhere, the sediments belong to the Lower Permian, or perhaps may be better classified under the broader designation Permo-Carboniferous. The Shihhotse series is also difficult to place. For example, a type of frond which is essentially Mesozoic, *Cladophlebis Nystrœmia* is to be found in the lower part of the division in considerable numbers. Another Mesozoic type is a species of *Dioonites, D. densinervis*. The presence of the following species, however, *Sphenophyllum Thonii, S. Thonii* var. *minor, Tœniopteris multinervis*, suggests that the beds are of Permian age, while Carboniferous forms, such as *Calamites Suckowii, Sphenophyllum emarginatum, S. Costæ*, and *Stigmaria ficoides*, have also been found. *Stigmaria ficoides* extends throughout the whole of the Carboniferous, but it is also to be found in the Permian. The most serious objection to placing the Lower Shihhotse flora in the Lower Permian is the purely negative evidence presented by the absence of any species of *Callipteris*. Its absence, however, may be an edaphical or phyto-geographical feature, as it is extremely scarce, and even sometimes quite lacking in the typical Lower Permian floras of Saxony and Bohemia. From the conflicting nature of the evidence, Halle has come to the conclusion that it is best to regard the base of the Shihhotse series as representing the beginning of the Permian in central Shansi, and that the beds possibly extend as far as the beginning

of the Mesozoic, although the latter possibility is largely discounted by the fact that the beds are not of sufficient thickness (450 m.), and of this thickness, 250 m. from the upper portion contains five genera of Mesozoic aspect. On the whole, it cannot be said that the Upper Shihhotse series reaches anywhere near the Permo-Triassic boundary, and at the present time there is no evidence that it extends anywhere above the middle of the Permian, and it is even possible that it may fall entirely within the Lower Permian. Again, if the Upper Shihhotse series represents the passage to the Triassic, the whole of the Shihchienfeng series would have to be included in the Triassic. But if, on the other hand, the Upper Shihhotse series be included in the Lower Permian, the Shihchienfeng series, though wholly continental, might be compared with the Permian Zechstein of Europe, which also marks a period of arid conditions. In such circumstances the change from humid to arid conditions would have more or less synchronised in Europe and Eastern Asia.

Geographical Relationship of the Flora. In the following table Halle has compared the plants found in central Shansi with those occurring in Europe and North America. The occurrence of identical forms is marked with a plus sign, of similar but this identical forms with a number referring to the name of a similar cause species in the right-hand column.

far as
wn in

TABLE IV

...si.	Europe.	N. America.	
		(+ ? 1)	(1) *Annularia ? maxima,* SCHENK, WHITE, 1912.
		' —)	
		⊦)	
		-)	(2) *Annularia radiiformis,* (WEISS). GRAND'EURY.
		-)	
		-)	
		-)	
		-)	

TABLE IV.—*Continued.*

Species from Central Shansi.	Europe.	N. America.
Sphenophyllum *oblongifolium*	(+)	(+)
,, *Thonii*	(+)	(+)
,, var. *minor*	(+)	(−)
,, *Costœ*	(+)	(−)
Bowmanites	(+)	(?)
Sphenopteris	(+)	(+)
Oligocarpa	(+)	(+)
Pecopteris (*Asterotheca*) orientalis.	(4)	(4)
,, *arcuata*	(5)	(5)
,, *Wongii*	(6)	(6)
,, (*Ptychocarpus*) unita.	(+)	(+)
,, *feminœformis*	(+)	(+)
,, (*Asterotheca*) hemitelioides.	(+)	(+)
Cladophlebis	(+)	(+)
Alethopteris	(+)	(+)
Callipteridium	(+)	(+)
Odonopteris *subcrenulata*	(+)	(+)
...mplectopteris *triangularis*	(−)	(7)
...uropteris	(+)	(+)
...toblechnum	(−)	(+)
spe...ropteridium	(+)	...8)
T...nopteris *multinervis*	(+)	
,, *Schenki*	(+)	
,, *Tingii*	(−...1)	(+)
,, *densissima*	(1...−)	(13)
Gigantopteris	...(−)	
,, *Whitei*	(+)	
Lepidodendron	(+)	

(4) *P.* (*Asterotheca*) *oreop-teridia* (SCHLOTH) STERNB.
(5) *P. pilosa*, STERZEL.
(6) *P.* (*Asterotheca*) *Miltoni* (ARTIS) BRGN.

(7) *Lescuropteris* M... (LESQ.) SCHIMP...

Cf. WHITE...

(10) *T.* ...DS...

...coriacea, SELL-
...fallax, GOEPP.
...Smithii, LESQ.
...ericana, WHITE.

...e, 1912, P. 511.

(17) *P. Planchardi*, REN.

Stigmaria *ficoides*
Cordaites *principalis*
Baiera
Saportœa
Dioonites
P...

...ere found to be common to
...seven similar species; wh...
America, and there we...

similar species. Although there are more species common to this region and Europe than to North America, the difference is not very great, and closely comparable, though not identified species, are found in greater number in North America and central Shansi. There is a further feature which makes the similarity to the American flora more striking. This is the occurrence of peculiar generic types which are only to be found in North America

...icotianæfo...
...ond to show *refe...*
...Ialle.)

...*lia* from Shansi, China. (1). En—
...venation. (2). Apical portion of

FIG. 20.—
large...amely, *Gigantopte...*
frond ...rites.

and eastern A...nus which may be d...*ris, Saportæa, Protoblechnum,*
and possibly ... different genera...
Gigantopteris...elii and probably *G. dentata*; and the other
probably repr... *Gigantopteris* is a form possessed of
G. nicotianæfolia, ...hem 30 cm. broad, pinnate, with a
G. americana and ...s pinnatipartite in its lower part
very large fronds ...owards the apex. The pinnæ are
large terminal se...osite, touching each other laterally
and becomes alm...ing an angle of 40 degrees to 80
generally opposite...
or slightly overlapp...

degrees with the main rachis, sessile, with the base often articulate. The margin of the pinnæ are dentate, sinuate or almost entire, the teeth corresponding each to one secondary vein and projecting in the direction of the vein, and often sharply pointed. The midrib is strong and reaches to the apex. *G. nicotianæfolia* and *G. Whitei* are shown in Figs. 20 and 21.

The first section of *Gigantopteris* was first described from, and is only known in Asia, while the type of the second (*G. americana*) was described by White from western North America. As long as each section was only known from one continent, the distribution of this form might have been thought to have been of no importance, but the discovery in China of *G. Whitei*, which is very closely related to, and may even be possibly the same species as *G. americana*, shows that White's original deductions as to the relations between the floras of North America and China were essentially

Fig. 21.—*Gigantopteris Whitei*, from Shansi, China. (1). Part of frond. (2). Enlarged portion of (1) to show venation. (After Halle.)

sound. Again, the rare genus *Saportæa* is only to be found in one or two regions in the Permian of North America, and the Chinese species may well be identical with the genotype.

Taken as a whole, the Upper Palæozoic flora of central Shansi definitely related to the contemporaneous floras of both Europe and North America in much the same manner as the two latter are related to each other. But in the upper part of the serie

the flora is distinctly more closely related to that of North America than to that of Europe.

It would be expected, in view of the short distance separating the Shansi flora from the Kusnezk flora (see above), that they would show a close relationship, yet the surprising result is that no such expected relationship is to be found. Only one species is common to both, *Pecopteris anthriscifolia*, a form which had a very wide distribution. Even closely related or at least similar species are but few. In fact, it may be said that the two floras are entirely different.

There are two explanations that may account for this remarkable state of affairs, in which two floras so close in time and space are so utterly different in their character : the variation may be due to the difference in the geological age of the floras, or it may be due to phyto-geographical factors. There is little doubt that the flora found in central Shansi is older than the Kusnezk series ; but, even in these circumstances, there is reason to suspect that phyto-geographical conditions also played a part, and there is a strong possibility that the Kusnezk flora and the *Gigantopteris* flora of central Shansi belonged to regions marked by strong differences in climate towards the close of the Palæozoic. Neither does the *Gigantopteris* flora appear to be related to the Indian Gondwana flora. In no case has a single species been found which is unquestionably identical with the *Glossopteris* flora.

In conclusion, it can be said that this flora of central Shansi formed part of the circumpolar Arcto-Carboniferous flora, and in its earlier phase only differed from the contemporaneous floras of Europe and North America in much the same degree as these differed from each other. Later, however, the upward increase of the characteristic East Asiatic element, the *Gigantopteris* flora, was probably due to a gradual fall in the humidity of the climate, leading eventually to the arid conditions which can be traced in the uppermost beds, *i.e.*, the Shihchienfeng series. This change in the climate more or less synchronised with similar changes in Europe and North America, but in southern China the *Gigantopteris* flora occurs in coal-bearing sediments.

Space will not permit of a discussion here of recent work on the Cainozoic flora. The investigations concerned with this side of the subject have been made mainly with the view to ascertaining the distribution of the flora, rather than with phyletic speculations. For a most interesting and very readable account to the recent flora, as well as that of the Mesozoic and Palæozoic, the reader is referred to the publication by Professor Seward, *Plant Life through the Ages.*

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CHAPTER III

EXPERIMENTAL METHODS IN RELATION TO THE SPECIES PROBLEM

Linnæan Conception of Species—Darwinian View—Jordani [...]
Species—Mutation Theory—Lotsy and Hybridisation—Genecolo [...]
—Classificatory Units.

A SATISFACTORY definition of the term " species " has alwa[...]
presented the utmost difficulty to biologists. From the poi[...]
of view of Linnæus, the matter was relatively simple. At tha[...]
time the conception of evolution of living organisms was no[...]
accepted, and he was able to recognise " just as many species a[...]
there were different forms created in the beginning." This vie[...]
was still further elaborated by him at a later date : " Species an[...]
all those diverse forms which the Infinite Being produced in the[...]
beginning, each of these forms has produced, in accordance wit[...]
the laws of generation, more like unto itself. Hence there are a[...]
many species as there are at the present day different forms an[...]
structures." Even to Linnæus, however, " varieties " presente[...]
a special problem, but this was surmounted by a further aphorism[...]
" A variety is a plant transformed by an incident cause such a[...]
climate, soil, heat, wind, etc."

The publication of the *Origin of Species*, by Charles Darwin, cu[...]
the ground from beneath the Linnæan position. It should be borne[...]
in mind, however, that it was only in the final form that the[...]
Darwinian theory denied the existence of Linnæan species as[...]
definite units differing in kind from a variety or individual[...]
Darwin himself recognised two kinds of variations : fluctuatin[...]
variations, which are now termed modifications, and chance o[...]
single variations, to which the name mutation has been given[...]
natural selection could act on either. Later, possibly as a resul[...]
of the attitude of Alfred Wallace, Darwin pinned his faith t[...]
individual variations as being the most promising material fo[...]

the action of Natural Selection. The logical sequel of Darwin's attitude to variation led him to deny the existence of species as a separate grade, distinct from a variety or even individual, and this view is categorically so stated in Chapter **II.** of the *Origin of Species :* " Certainly no clear line of demarcation has as yet been drawn between species and sub-species—that is, the forms which in the opinion of some naturalists come very near to, but do not quite arrive at, the rank of species : or, again, between sub-species and well-marked varieties, or between lesser varieties and individual differences. These differences blend into each other by an insensible series ; and a series impresses the mind with the idea of an actual passage. Hence I look at individual differences, though of small interest to the systematist, as of the highest importance for us, as being the first steps towards such slight varieties as are barely thought worth recording in works on natural history. And I look at varieties which are in any degree more distinct and permanent, as steps towards more strongly-marked and permanent varieties ; and at the latter, as leading to sub-species, and then to species."

Nevertheless, elsewhere (Chapter VI.) he states : " To sum up, I believe that species come to be tolerably well-defined objects, and do not at any one period present an inextricable chaos of varying and intermediate links " ; and he goes on to elaborate the view that this relative distinctiveness of species may be due to the extinction of intermediate varieties.

The Linnæan concept of species was also attacked by Alexis Jordan, but from quite a different standpoint from that taken up by Darwin. In the first place, Jordan was not a believer in evolution, and, secondly, he was a determined antagonist of the Darwinian theory. But from cultural experiments, combined with a careful and minute morphological examination, he claimed to have proved that many Linnæan species were not homogeneous units, but were composed of a number of smaller units. Here he attack on the Linnæan position was not from any question of the immutability of species, but was concerned with the rank of the immutable units. Jordan was that *bête noire* of taxonomists —a " species splitter."

It is not necessary to discuss the experiments of Mendel here but three important points from this work need emphasis connection with the species problem. The first is that " new forms may arise from a cross through a redistribution of parent characters; secondly, the heritable characteristics of a race not constitute an indivisible whole, but can be analysed in unit-characters, or, as they are now called, " factors " or " genes and that these can be inherited independently of one another thirdly, that the plant as defined by its somatic character (phenotype) and the hereditary type to which the plant belong (genotype) are two different things.

The questions arise here: Is the Linnæan species a real entity or merely the conventional grouping of genuine units? and secondly what are the actual units upon which evolution works?

In 1901 de Vries laid stress upon the fact that modifications are not inherited, and therefore provide no basis for artificial or natural selection. But among cultivated plants, " sports " or mutations are often found, and these differences can be passed on to the progeny. The plant upon which de Vries based his conclusions, *Œnothera Lamarckiana*, has since been shown to be a hybrid, but a number of recognised mutations have been known for a long time, e.g., *Chelidonium laciniatum*, which was discovered in 1590 by Sprenger, and differs from *Ch. majus*, L., in its laciniated leaves and petals. There is also the classical case of the " runnerless " or " Gaillon " strawberry, a runnerless form of *Fragaria alpina*. The unfortunate collapse of the chief corner-stone of the de Vries structure (*Œ. Lamarckiana*) brought the mutation theory of evolution into considerable disrepute, especially among those who regarded hybridisation as more than a sufficient source of new species.

The chief protagonist of the hybridisation theory of evolution is Lotsy, and his views are given in a short book, *Evolution by Means of Hybridisation*. Lotsy rejects the Linnæan species being no species at all, and at the same time he a*y* as species as Jordanian species, since breeding true to type ed his rejects the a proof of genetic purity. His own definit ising mate necessari

" A group of individuals of identical constitution, unable to form more than one kind of gametes." But this definition is by no means entirely satisfactory, as it denies the possibility of muta-tions. There is also the further difficulty that though perfect homozygotes and genetically pure races are laudible concepts, there is considerable doubt whether such ideal individuals or races have ever been isolated.

Two other workers (A. L. and A. C. Hagedoorn) have put forward arguments very similar to those of Lotsy, but they differ from him in that they do not deny the possibility of muta-tion, but consider that it does not play an appreciable part in evolution ; they also make much use of the concept " potential variability." Thus, the total potential variability of either an individual or group is measured by the number of genes for which that individual or group is not homozygous. Isolation is supposed to be the main agency causing a reduction of the total potential variability, and not selection, as Darwin supposed.

Comparison of the views of Lotsy and the Hagedoorns towards Linnæan species is interesting. It has already been stated that Lotsy denied the existence of Linnæan species. The Hagedoorns, on the other hand, state their views as follows : " A species is a group of organisms so situated and so constituted that it tends automatically to reduce its total potential variability, and which for this reason tends to become pure for one specific type." And, again : " Species are realities and they are stable, not changing. Further, we believe that those individuals which are seen to differ in one striking point from the members of a species among which they live constitute a variety, whereas individuals differing in a group of characters from hitherto described species constitute a new species."

One important point emerges from this work, namely, that the evidence upon which these workers base their views shows the remarkable distinctness and relative purity of local or spatially isolated races ; therefore isolation as a factor in species formation is now taking an important position in the literature.

Turesson (1922), under the title " Genecology," has advanced a number of important arguments on this subject, and it will be

when exposed to the more or less extreme conditions of the natural habitat. The external result is an apparently homogeneous population of dwarfs known as *S. pratensis* f. *nana*. When cultured, however, this apparently homogeneous population breaks up into its component parts, and the great hereditary variation as to height is shown.

Two special cases require consideration here, *Atriplex* and *Hieracium*. *Atriplex* is especially suited for an investigation of this nature. All the species are annuals and have been long known to be exceptionally polymorphic.

Atriplex litorale is a common species along the Swedish coast. Its root-system is shallow and the plants are more usually limited to the lower reaches of the beach. Further up the beach dwarf forms are produced. The species reaches its greatest development in sheltered positions, especially along beach-lines, coves, etc. Collections of small plants and seeds were made and grown in culture. It was found that the population was made up of a large number of biotypes, often differing noticeably from each other. In the first cultures that were isolated seventeen biotypes were distinguished, and in none of them was a similar combination of characters found. In some only small differences were to be seen, but these differences repeated themselves with absolute exactness every year.

Atriplex sarcophyllum is one of the most distinct species of the genus. It is distributed practically along the whole length of the coast of Sweden, occupying the zone nearest to the water, in which no other species of *Atriplex* thrives at all well. *A. sarcophyllum* flowers early (May and June), and seeds and plants were collected from thirty different localities. Some remarkable geographical variations of a hereditary nature were found to occur. For example, it was found that the eastern coast-line was inhabited by a certain group of forms distinctly different from the population of the Sound region, and both these forms differed from those inhabiting the western coast-line. It will be convenient to discuss these various geographical groups separately.

Taking first those found on the eastern coast-line, it was

observed that the plants of the whole group were thick and
fleshy, with rhombic-lanceolate leaves, long unbranched and, in
the upper portion, leafless inflorescences with the basal branches
ascending. In the field this group showed great uniformity of
appearance, but on cultivation it was found to break up into a
number of different forms, and the offspring obtained from these
isolated forms agreed in all details with the parent plants. It
was also ascertained that the individuals of these cultivated
biotypes differed markedly in one respect from the plants growing

FIG. 24.—*Atriplex sarcophyllum* from the east coast (Oxelösund).
(After Turesson, *Hereditas*.)

under natural conditions. The " wild " forms were dwarfs
compared with the plants obtained in culture. Two biotypes
isolated from cultures are shown in Figs. 24 and 25.

In the case of plants growing in the Sound region, it was found
that there was a small form growing along the marshy shore-
line at Limhamn, near Malmö. It flowered in May and had
thicker leaves than other forms of the genus growing in the same
place. A number of plants were collected from this region and
brought into cultivation. They resembled the forms from the
eastern coast-line in being early flowering and in the possession of
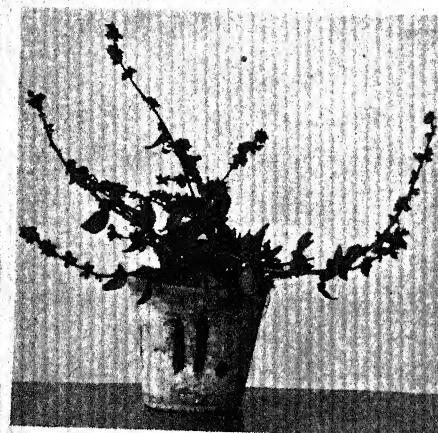
FIG. 25.—*Atriplex sarcophyllum* from the east coast. (Gottland). (After Turesson, *Hereditas*.)

thick, fleshy leaves, and also with regard to the shape of the leaves as well as of the inflorescences. There were, however, also a number of important differences. Towards maturity, the oldest fruiting bractlets in the inflorescences of these forms became large and leafy and their petioles elongated, a characteristic to be found in the plants growing in their natural habitat. The branches were also often procumbent and even prostrate. Figs. 26 and 27 show an ascending-erect biotype and a prostrate form respectively; both plants were isolated and pure-cultured from the original Limhamn material.

The type along the western coast-line showed great hereditary

FIG. 26.—*Atriplex sarcophyllum* from the Sound. Ascending-erect form. (After Turesson, *Hereditas*.)

variation. Individuals with leafy, fruiting bractlets with long stalks were entirely absent and showed a resemblance to the
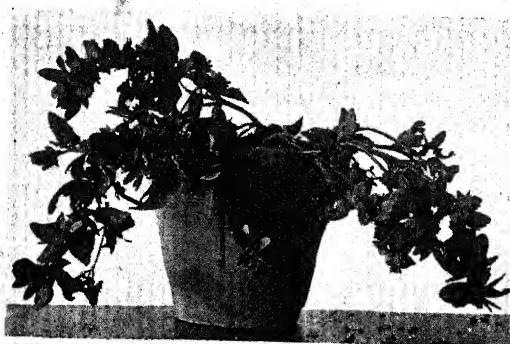
FIG. 27.—*Atriplex sarcophyllum* from the Sound. Prostrate form. (After Turesson, *Hereditas*.)

fruiting bractlets of plants from the east coast, but the branching habit is different. They were usually more branched from the
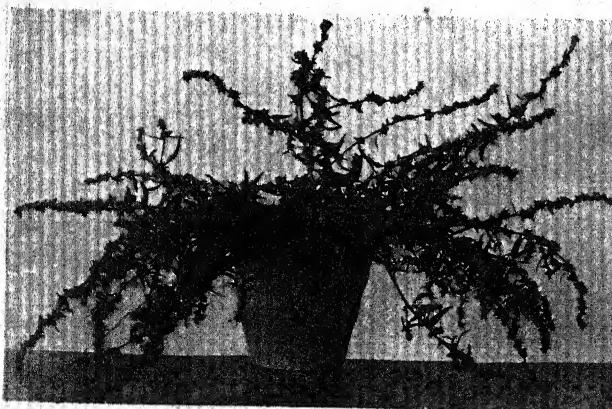
FIG. 28.—*Atriplex sarcophyllum* from the west coast. Ascending-erect form. (After Turesson, *Hereditas*.)

base, and most often prostrate both as to main axis as well as to basal branches. Again, a number of biotypes were isolated; some of these differed in a number of particulars, but were quite

definite within limits. They differed from the eastern type all being prostrate, and the time of flowering was a little later than either of the forms obtained from the eastern coast-line and from the Sound. Figs. 28 and 29 illustrate biotypes from this region.

Hieracium umbellatum, L., is also a species which shows wide variations, and two types occur along the north-west coast-line of Sweden which differ from the inland form. The latter is a tall, erect plant, with lanceolate leaves and rather contracted inflorescence, and is a typical woodland form. The two types found on the coast-line are restricted to two different and distinct
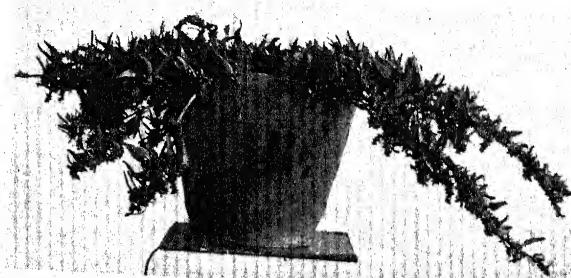
FIG. 29.—*Atriplex sarcophyllum* from the west coast. Prostrate form. (After Turesson, *Hereditas*.)

habitats: one occupying rocky and much exposed points, the other growing on sand dunes. Both the cliff form and the sand-dune type are more or less prostrate, and possess rather narrower leaves and more contracted inflorescences than the inland form. Summarising the results, it can be said that the hereditary variation shown within the species is marked, and that variations occurring within the limited geographical area dealt with roughly group themselves into five classes :—

1. *The Dune Type of the Shifting Dunes* from the drift-sand, and white dunes along the western coast and the Sandhammar region. This type comprises individuals with rather narrow leaves and slender, less erect, and sometimes more or less prostrate stems. The power of shoot regeneration is very marked during

the autumn. The fruiting stage is reached in the early part of September.

2. *The Dune Type of the Arenaceous Fields* and stationary dunes from the eastern coast-line. The individuals of this type closely resemble the dune form of the shifting dunes, but differ in the extreme prostrateness of their stems. The power of shoot regeneration is less marked, and is often entirely wanting.

3. *The Sea-cliff Type of the West Coast.* This type comprises broad-leaved individuals, more or less prostrate. The cells of the leaves are considerably distended, and the growth-form of the plants is contracted and bushy. The fruiting stage is first reached in the latter part of September or the beginning of October.

4. *The Sea-cliff Type of the East Coast.* This resembles the preceding form as to the breadth and thickness of the leaves. The growth-form, however, is quite different; the plants are tall and almost as erect as the inland type. The fruiting stage is reached at the same time as Group 3.

5. *The Woodland Type of the Interior.* This form comprises stout, erect plants with lanceolate leaves. The leaves are tough, but considerably thinner than the leaves of the preceding types that have been described, and possess only two or, at the most, three layers of palisade cells. The inflorescences are generally umbelloid and contracted. The fruiting stage is reached in September.

It will be seen, from the various examples cited above, that although observed characteristics were in certain cases purely modificatory in nature (*Lysimachia vulgaris*), in the vast majority of cases there was a differentiation of the species-population into different hereditary variations in the different habitats.

There is also the further fact to be considered, that in the strictly intermediate area between two habitats of different nature and populated by two different types, no intermediate form is to be discovered, but, on the contrary, individuals of the two different types and hybrids between them make their appearance.

Differentiation of plant species into different hereditary habitat

istics, which remained constant under cultivation. The wholly viviparous class may, late in the season, develop a few short and weak inflorescences bearing normal flowers and seeds, although under natural conditions it is doubtful whether these help in the spread of the plant. The partially viviparous forms, on the other hand, normally develop one to many flowers in the base of the spikelet, and a bulbil above, and a number of gradations are to be seen between the wholly sexual and the wholly viviparous classes, but these intergrades keep constant in culture.

Vivipary includes a number of different phenomena, and may be either *modificatory* and can be induced, as in *Phleum pratense* and perhaps the majority of grasses, or it may be *hereditary.* In those grasses in which vivipary is of ecological significance, two groups may be distinguished : (1) those in which the genotype factors involved are of no ecological significance (*Zea mays*), and (2) those in which the genotype factors involved in bringing about vivipary have led to the formation of successful habitat races, as in *Festuca*, *Poa* and *Aira*. The situation is analogous to the apomictic species in *Alchemilla*, *Hieracium* and *Taraxicum*, and occasionally, like these, they have a limited distribution. The partially viviparous forms correspond to the condition found in *Pilosella*, in which apomixis of the apospory type does not exclude facultative sexuality.

According to Turesson these apomictic types represent nothing but biotypes. fixed as to morphological details and multiplied through apomixis. Apart from reproduction, there are no principal differences between the biotypes of apomictic groups and those that reproduce exclusively by sexual means. On these grounds, therefore, he has introduced some fresh terminology, thus : a form (= biotype) which is apomictically reproduced (through apogamy and vegetative propagation) is called a *forma apomicta* or *apomict* (abbreviated to *apm.* before the type name). A form (= biotype) which is *obligate* sexual, and can therefore interbreed to the greatest extent with other types, is called a *forma amphimicta* or *amphimict* (abbreviated to *amph.* before the type name). A form (= biotype) which, besides apomixis, can reproduce itself sexually (facultative sexuality), as in the

within *Poa alpina* favours, to a certain extent, the view that the alpine ecotypes of this species immigrated into Scandinavia during the *Dryas* period, and that the lowland ecotype entered independently and possibly at a later date when the climate was warmer. On such a view, the glacial relic hypothesis for the presence of these plants becomes no longer tenable. The rigorous selection to which the first populations of immigrating *Poa alpina* must have been subjected, together with the simultaneous elimination of biotypes which were not sufficiently hardy, must have thinned these populations to such an extent that subsequent differentiation of a lowland ecotype from this selected material has been impossible.

If *Poa alpina* be a specialised ecotype in the lowlands, it can be understood why this form has not reached the wide distribution of the alpine biotype, since glaciation must have meant great destruction to lowland forms, while the alpine types would have been able to find shelter in ice-free refuges. In other cases, lowland ecotypes of other species-populations, even if not entirely destroyed, became through migration much scattered and depauperated with regard to their biotype contents, and are at present only represented by Arctic and alpine ecotypes (*e.g.,* *Dryas octopetala*, *Loiseleuria procumbens* and *Oxyria digyna*). It is probable that it is to this group that *P. alpina* belongs. Its lowland ecotype in Sweden is very localised in its distribution, and this rarity is possibly due to the elimination of a great part of its original biotype components.

The correlation of plant and habitat, *i.e.,* the effect of climatic factors on ecotypes, has been statistically examined by Turesson for a number of species. The two characters which were most easily measured and, at the same time, were of most ecological importance were height and earliness. The material was obtained from different parts of Europe, and also from Siberia.

Only a few examples of the results can be discussed here. Apart from the alpine types, the material investigated came from four main regions, differing widely from one another with regard to climate. From the number of observations that were made, it was clear that climate strongly influences the distribution of

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the biotypes within a species. Taking first the group of æstival plants (twenty-five species in all), it was found that in the northern part of the investigated area pronounced earliness was combined with moderate height, whereas in the southern parts of the area, lateness was combined with great height. Again, in the extreme maritime parts of the west, low growth was combined with lateness, whereas in the east, great height was combined with earliness. It was also found that from the various seedling generations which were grown from alpine and lowl types of the same species, the former are genotypically lower height and earlier than the latter.

Corresponding to the change in climate, there is evidently a change in the genotypical composition of the species-population. Thus early and low-growing types are perhaps best suited for the short and relatively cool summer of the north of Europe, whereas the longer vegetative season and higher summer temperature in the south apparently favours more luxuriant growth and a late-flowering type of species. In the western maritime regions lateness of flowering was found to be characteristic of the species (*e.g.*, in *Fragaria vesca*, *Geranium silvaticum*, *Geum rivale* and *Spiræa ulmaria*), but farther west, with greater intensification of this type of climate (as in the Færöes), lateness becomes combined with the further feature of low growth, or even of dwarf forms. On the other hand, the continental climate in the east, with a fair amount of summer rainfall, supports types in which great height is combined with earliness.

To consider the case of spring plants, which were found to differ from the æstival forms with regard to earliness, the behaviour of *Caltha palustris* will serve as an example. In the forms from South Germany, earliness of flowering was found to be more pronounced than in the types from South Sweden, but still further north, earlier types reappeared once more. Whether this reappearance of earliness also applies to other spring plants is at present unknown, but it is very probable. As a rule the floral buds of spring plants hibernate in a more or less advanced state. *Caltha palustris*, for instance, has all parts of the flower bud well developed in autumn. It therefore seems reasonable to

suppose that types with particularly advanced floral winter have been selected in the south and extreme north. In the so the leafing of trees, as well as the awakening of vegetation whole, takes place at an earlier date than in regions far north, and the southern spring plant must of necessity be ear than more northern forms in order to conform with the periodicity. In the extreme north the shortness of the vegetation period obviously calls for a similarly well-advanced floral structure.

The case of *Plantago maritima* has been investigated by Gregor (1920) with regard to the effect of environment on isolated wild populations of this plant. The distribution of *P. maritima* in this country is continuous throughout the coastal regions, but inland it is scattered and mainly confined to high altitudes.

It is at present not clear whether the flora of the British Isles was completely destroyed during the Pleistocene, or whether some portion survived in the South of England during the period of maximum glaciation. In Scotland, however, it is certain that the greater part was denuded of its previous flora, and the present flora was established with the retreat of the ice and the onset of more congenial climatic conditions. For a discussion of the lines of plant invasion into Britain from the Continent, consult Matthews, Chevalier, Turrill and Woodhead.

It is, however, the climatic conditions prevailing during the maximum period of glaciation that are of importance in this connection, and whether or not *P. maritima* survived the extreme conditions of that time. It is probable that the inland distribution of *P. maritima* is a remnant of a previous more general distribution, and that this species followed the retreating ice at the close of the glacial period. Owing to its xerophytic nature, it would be well adapted to withstand the prevailing conditions of physiological drought. Migration of *P. maritima* into this country was presumably from a Continental stock at the close of the Pleistocene and subsequent environmental conditions are responsible for its present restricted distribution in inland regions. Apparently competition has been the main factor in driving it out from intermediate positions, since it can grow quite well under mesophytic conditions at low altitudes both in culture and also in the

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This fact bears out Salisbury's statement, that plants v not where they would, but where they must. The species of *P. maritima* includes many growth-forms which e from the decumbent to the erect. Under natural conditions plants forming a habitat population may sometimes be so modified by extreme environmental conditions that they assume a phenotypic similarity which masks the presence of the various forms, and it is only when such plants are brought into more congenial surroundings that the different types in the population become evident.

The plants used by Gregor came from a single locality on the east coast of Scotland, and, though restricted in area, this region was divided ecologically into two regions: exposed rock immediately above the high-tide mark, and an adjoining grassy slope. From a comparison of leaf height and scape height of the two populations, it became clear that there was a decided tendency towards lower growth in the case of the rock population. Under natural conditions, one of the most marked characteristics between the two populations was leaf form. The leaves of the rock form were short and cylindrical, whereas those of the plants

FIG.—*Plantago maritima*. The upper figure is specimen collected from exposed rock habitat and the lower figure shows the same plant in culture. (After Gregor, *J. Gen.*)

slope were long and dorsiventral, and exhibited obvious signs of etiolation. Plants which had been taken from the rock habitat and cultivated for a year lost their characteristic cylindrical leaves and now assumed the dorsiventral type (Fig.). Observations on the floral characters, time of flowering, leaf shape, and the presence or absence of hairs on both leaves and scape, though showing considerable variation, gave no definite distinction between the two populations.

The two populations under discussion are an example of popula-

tical differentiation unaccompanied by spatial isolation. Under natural conditions they occupied two sharply differentiated adjoining areas, and the environment had modified both populations considerably, but the effect of environment was more evident on the rock forms, and these latter in cultivation showed a larger proportion of low-growing forms than plants obtained from the grass habitat; nevertheless, the grass forms contained no classified type which did not also occur among the rock forms. It is possible that occasionally phenotypical characters (here dwarf habit) determine the survival of certain forms, and one is therefore led to attach some importance to the value of any genotype as represented by a particular phenotype.

Collins, and also Du Rietz, have advanced a number of criticisms against the genecological views of Turesson. Collins' criticisms need not be detailed here, as he has misinterpreted Turesson's statements, and accuses the latter of Lamarckianism, but Turesson has never claimed to be a Lamarckian, and, in point of fact, expressly denies that his results can be explained by Lamarckianism.

Du Rietz's paper is really an attempt to reconcile old terms with modern experimental data, and the result is not very successful. According to him, the fundamental principle in the grouping of individuals into taxonomic units of different rank is "the lines or zones of discontinuity formed in the series of biotypes a geographical, ecological and physiological isolations." Geographical isolation, however, is clearly the most important to Du Rietz. Turesson holds the opposite view, namely, that it is the genotypical constitution of the individual that is of the greatest importance for a natural grouping, and therefore physiological and ecological isolation is of far more importance in this connection than a mere geographical barrier.

Du Rietz defines a species thus : " The smallest natural populations permanently separated from each other by a distinct discontinuity in the series of biotypes, are called species. A species thus is a population consisting either of one strictly asexual and vital biotype, or of a group of practically indistinguishable strictly asexual and vital biotypes, or of many sexually propa

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ing biotypes forming a syngameon separated from all other
a more or less complete sexual isolation or by comparati
small transitional populations.''

As Müntzing, Tedin and Turesson have pointed out, '' se
isolation '' is an extensive concept to use, since, according to
Rietz, it may be either geographical or merely physiological.
the former case the species may be perfectly fertile *inter*
Again, morphological differences between two geographica
isolated populations do not need to be great, and if individu
differ slightly, but nevertheless constantly, from each oth
some taxonomists will treat these as varieties or sub-spec
while others will treat them as separate species. But Müntzin
Tedin and Turesson claim that if a species population has
some reason become separated into two or more geographica
isolated parts, and if these parts differ from one another in slig
morphological features and give fertile and vital hybrids whe
artificially crossed, this is not in itself a sufficient argument
raise these '' part-populations '' to the rank of species, and the
must be considered as units of lower than specific rank, and mus
be placed in the same species because of their genotypica
similarity.

Du Rietz also attempts to define the terms sub-species and
variety : '' a sub-species is a population of several biotypes
forming a more or less distinct regional facies of a species ''
while a variety is defined as '' a population of one or several
biotypes, forming a more or less distinct local facies of a species.''
It is somewhat difficult to draw a clear distinction between
'' regional '' and '' local,'' and a geographical criterion is therefore
a rather vague and unsatisfactory method of distinguishing
between sub-species and variety.

Müntzing, Tedin and Turesson's criticism of Du Rietz's defini-
tions of taxonomical units can best be given in their own words :
'' They leave the internal relationship and the reaction between
genotypical constitution and environment quite out of the question.
Even with these sacrifices, however, they do not adequately
limit species, or sub-species from varieties.''

Gregor and Sansome (1927) have made a number of interesting

might be suggested that this differentiation was due to isolation or seclusion, through barriers of various kinds, from the main alpine population. But the evidence is against such a view, because these plants differ from the alpine forms not only morphologically but also physiologically. On the other hand, distinguishable units were found within the lowland population; for example, lowland types gathered from Stockholm differed in certain morphological details from those gathered at Öland, and the interpretation of these two types as colonies within the lowland ecotype, which have maintained their respective morphological features on account of purely geographical isolation, may be fairly safe. Such types are termed by Turesson *seclusion* types. More recently Gregor (1931) has suggested the term *geo-ecotype* for forms which owe their characteristics to their geographical position.

The question arises as to whether œct. *pediacus* has been evolved from the alpine populations through selection of biotypes for lowland conditions. The first flora (*Dryas* flora) after the retreat of the ice also contained a number of water forms (*e.g.*, *Myriophyllum* and *Hippuris*), and the argument has been put forward that the climate was far from being Arctic. But on account of the very slight morphological differences between different climatical types of a species, conclusions from fossil data are not of a particularly reliable nature. Many of our common species are split up into a number of specialised climatical, hereditary types or ecotypes, a fact which seriously complicates the history of immigration of a species into a formerly glaciated region. It may well be that our common species had already been split up into ecotypes before the last glaciation. That alpine ecotypes immigrated into Scandinavia during the *Dryas* period, or possibly slightly later, is a feasible hypothesis, since climatical conditions at that period would have favoured such alpine ecotypes. On the other hand, the immigration of lowland ecotypes has in all probability taken place at a much later period, and during times when a suitable climate favoured such entry and spread of these forms.

The fact that different ecotypes have been found to exist

case of apospory, nucellar embryony, and vivipary is calle
forma amphi-apomicta or *amphiapomict* (before the type nam
aapm.).

It is clear that in biotype populations where sexual reproductic
is replaced by apomixis, the apomicts within such a populatio
cannot interbreed. Frequently extensive biotype-compoun
are built up exclusively of apomicts (*e.g., Alchemilla vulga*
and *Antennaria alpina*), and in these circumstances such com
pounds represent units of a different nature from those discusse
previously, and the term *agamospecies* is suggested to cover unit
of this nature.

Turesson has applied his methods and reasonings to the distribu
tion of alpine floras, especially in connection with the nature o
glacial relics. It has frequently been assumed that isolate
occurrence denotes antiquity. The distribution, for example, o
the alpines *Polygonum viviparum*, *Arctostaphylos alpina* and
Pedicularis sceptrum carolinum in the lowlands of Sweden wa
claimed to demonstrate that these were glacial relics left ove
from the period following immediately upon the melting of the
inland ice. It is now known that the occurrences of these plant
in the lowlands must be interpreted as recent immigrations

For the experimental testing of the relic hypothesi
alpina was selected from a number of different localit
Scandinavia, grown in culture and compared with true a
forms. The characters used for comparison were length a
width of sterile rosette leaves, length and width of uppermo
stem leaf, and length and number of flowers in the spikele
Comparison of the lowland plants with alpine forms showed tha
the Scandinavian population of *P. alpina* can be split into thr
different ecotypes : an alpine (œct. *alpinus*), a sub-alpine for
(œct. *subalpinus*) and a lowland ecotype (œct. *pediacus*). T
latter lowland form differs from the alpine and sub-alpine
both morphological and physiological features. For example,
water requirements are low, whereas œct. *alpinus* has a h
water requirement, and œct. *subalpinus* takes up an intermedia
position in this respect.

The distribution of œct. *pediacus* is very restricted, and

observations on the genetics of wild grasses in relation to their distribution. The majority of perennial grasses are self-sterile, whereas the annuals that were tested in this case were found to be self-fertile, though it does not necessarily follow that they are self-pollinated in Nature. The occurrence of self-sterility in a population is of importance in the study of wild populations, since in a highly self-sterile population the production of seed depends completely upon cross-pollination.

The examples studied were *Lolium perenne*, *Dactylis glomerata* and *Phleum pratense*, all of which are important agricultural grasses. The characters of *Lolium perenne* were found to be highly variable. Some of these characters were readily influenced by environment, and were therefore modificatory in nature, whilst others were not appreciably affected by environmental factors, and behaved as variations. The most extensive observations were made from plants obtained from a coastal area. The most pronounced feature of the plants from this region was their prostrate habit of growth. Although grown for two years at Corstorphine, and showing more vigorous growth than in their natural habitat, the plants still retained their low habit of growth and other characters. This prostrateness of form was at first thought to be due to the direct effect of the extremely close grazing by rabbits and sheep that the plants had been subjected to in their natural environment. Hand crosses were made between several similar phenotypes. The progeny were not uniform for the flat character, thus indicating that the parents were not all of the same genotype.

Since all the individuals collected from the one population showed the prostrate habit of growth, and when allowed to grow naturally, without cutting or grazing, they retained this habit, and, lastly, they were not all homozygous prostrates, selection of some kind must have made the population *phenotypically* alike.

The suggestion is put forward that this uniformity of phenotypes is due to the heavy grazing, and only genotypes which could produce like phenotypes under these conditions were able to survive and set seed. If these prostrate plants be compared with the erect commercial varieties, it is found that the great

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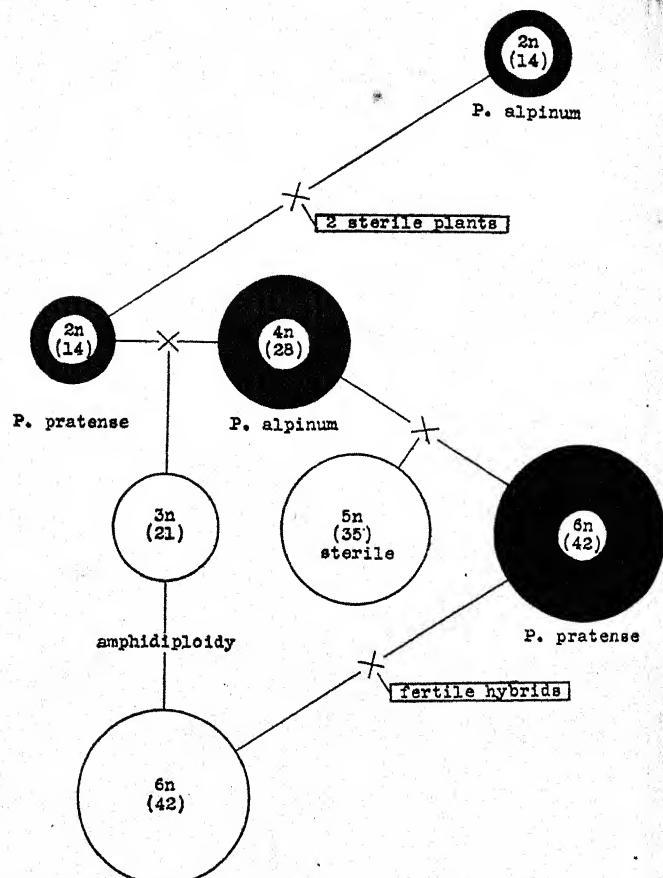
FIG. 32.—Diagrammatic representation of different crosses of *Phleum pratense* × *P. alpinum*.   (After Gregor, *N. Phyt.*)

The leaves are generally long and broad, and this group scarcely, if ever, is common on closely grazed pasture ground.

In the second group (*b*), the British " Wild " form, tillering was very prevalent, and it was a low-growing form compared with that described above.   The fertile stems were slender and hap corms were poorly developed, while in some plants they we completely absent.   This group is also distinguished from (*a*) by

smaller seed size, and leaf length and breadth were variable. In general terms, this group is suited to grazing conditions.

Groups of smaller magnitude were also distinguished within these two groups. The particular forms studied under uniform cultural conditions showed differences in growth-habit, *e.g.*, prostrate, decumbent, ascending and erect. Though of lesser magnitude, these groups are of ecological importance.

Reciprocal crossing was attempted between the two groups, and failed in all cases, and no seeds were obtained. In order to differentiate between the two *P. pratense* groups, crosses were made between members of each group and the species *P. alpinum*. No difficulty was encountered in obtaining hybrids from Group (*b*), *P. pratense* × *P. alpinum* (Scottish),[1] but in only one case was a hybrid obtained when Group (*a*) *P. pratense* × *P. alpinum* (Scottish) was crossed.

All the hybrids obtained from the mating Group (*b*) *P. pratense* × *P. alpinum* (Scottish) were found to be almost intra-sterile. Nevertheless, from some 500,000 unprotected flowers of F_1 plants growing in the experimental field, four seeds which germinated were obtained. The fertility of these four plants was as follows: (1) male and female sterile; (2) some normal pollen grains produced, female sterile; (3) male sterile, comparatively female fertile; (4) pollen production equal to *P. pratense* and *P. alpinum*, comparatively female fertile.

When the F_1 progeny were back-crossed to *P. alpinum* (Scottish) only five hybrids were obtained, all of which were male and female sterile. The single hybrid obtained from the cross Group (*a*) *P. pratense* × *P. alpinum* (Scottish) failed to set seed, and the plant was found to be male sterile. Fig. 32 shows a diagrammatic representation of the various crosses.

The failure to obtain hybrids between Group (*a*) × Group (*b*) combined with the further fact that there is a marked differe... haviour when Group (*a*) × *P. alpinum* (Scottish) is crossed... (*b*) × *P. alpinum* (Scottish) is crossed, confirms the ... that

ould be mentioned that the Scottish form of *P. alpin*... for it... y from the Swedish type utilised, both as regards habit of g... osome number.

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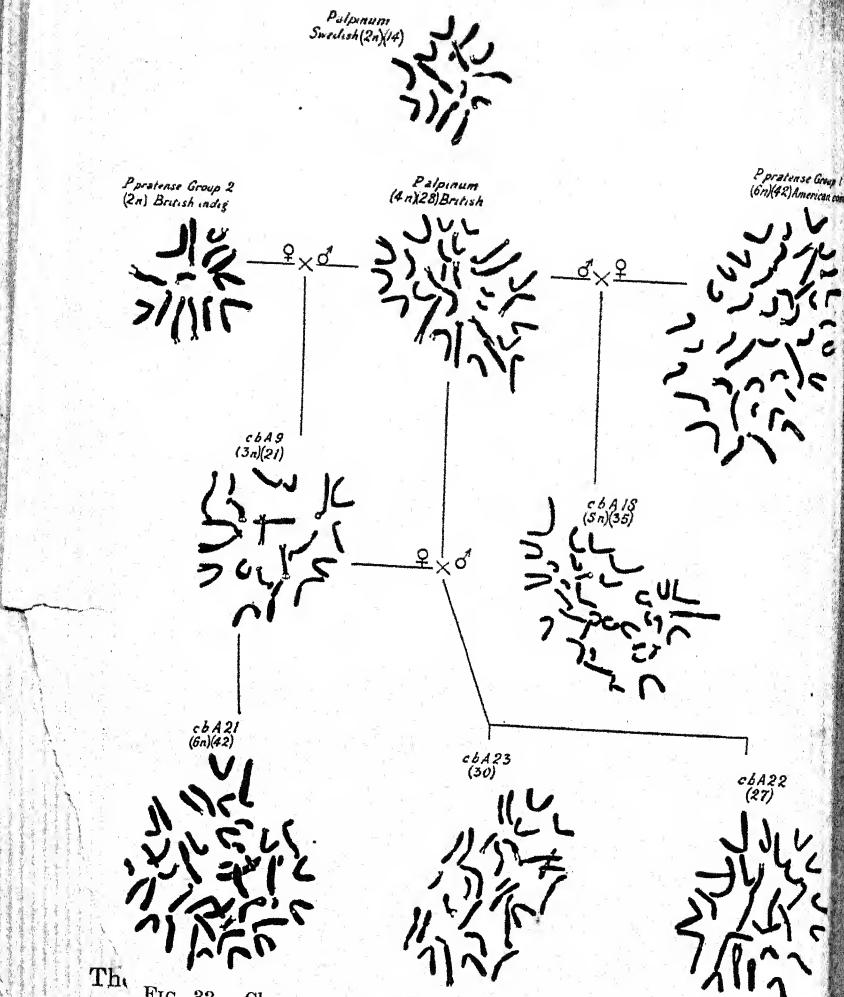
FIG. 33.—Chromosome complements of various crosses in *Phleum*.
(After Gregor & Sansome, *J. Gen.*)

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was v classification of *P. pratense* into two groups.   The cytologic
that des e bears on this point.
corms v is apparently the basic chromosome number in *Phleum*
c ompl romosome complements of the various crosses describe

above are shown in Fig. 33.   The cross between *P. pratense* $(2n)$ $\times$ *P. alpinum* $(4n)$ gave several plants with the $3n$ number of chromosomes.   These hybrids were partially sterile, but gave rise to four plants, three of which on examination proved to be hexaploids.   The triploid hybrid, however, was found to be more fertile when pollinated with *P. alpinum* $(4n)$, and several plants were obtained in which the chromosome number ranged from 26 to 30 (somatic).   It is probable that the hexaploids arose from the fusion of gametes having the somatic $(3n)$ number of chromosomes.   In such a hybrid as the *Phleum* triploid it is possible that in self-fertilisation a zygote is formed by gametes arising from restitution nuclei rather than by the normal process of meiosis, which in a triploid is irregular.   The reduction division in a triploid will give rise, as a general rule, to gametes with chromosome numbers which range from n to $2n$, but on occasion the chromosome number may be higher.

The cross *P. pratense* (Group (a) $(6n)$) $\times$ *P. alpinum* $(4n)$ was only obtained with difficulty.   The single plant obtained from this mating was completely sterile and a pentaploid, the pollen-mother-cells of which contained univalents, bivalents, and compound structures of higher valency at the heterotypic division of meiosis.

The cytological facts given above offer a fairly satisfactory interpretation of the behaviour of *P. pratense* in breeding and of its relationship to *P. alpinum*. The facts appear to show that the plants observed have a common phylogeny, and the fact that *P. alpinum* can be crossed with both Group (a) and Group (b), while these, up to the present, have not crossed with one another, is to be expected on cytological grounds.

The two groups within the Linnæan species *P. pratense* also exhibit different habitat preferences. Group (a) is mainly confined to moist habitats, whilst Group (b) is found principally in drier situations.   This type differentiation is further influenced by the prevalence of grazing animals.   It would seem that Group (a) cannot survive under severe grazing conditions, for it is seldom if ever to be found in old, heavily grazed pastures.

It has already been shown that it is possible to connect

P. alpinum (4*n*) with both forms (2*n* and 6*n*) of *P. pratense*, L., and to bridge the sterility gap between these forms by employing hexaploid individuals (see Fig. 32). It may therefore be concluded that the Linnæan species *P. alpinum* and *P. pratense* constitute a natural group, or, in Turesson's terminology, a cœnospecies, comprising several groups of lesser magnitude, ecospecies, and these contain still smaller units, ecotypes. The present position may be shown as follows (Gregor, 1931) :—

Cœnospecies

PHLEUM PRATENSE—ALPINUM

Ecospecies

P. pratense diploidium.	*P. alpinum diploidium.*
P. pratense hexaploidium.	*P. alpinum tetraploidium.*

Ecotypes

P. pratense 2*n* prostratum.		
,,	decumbens.	
,,	ascendens.	
,,	erectum.	(Ecotypes of *P. alpinum* also exist.)
,,	6*n* decumbens.	
,,	ascendens.	
,,	erectum.	

The genus *Galeopsis* has been studied both genetically and cytologically by Müntzing, and special attention was paid to the delimitation of species and polymorphism in the genus. *Galeopsis* is divided into two subgenera, subg. *Ladanum* and subg. *Tetrahit*. The former group contains the sub-species *G. Ladanum, G. angustifolia, G. pyrenaica, G. ochroleuca* and *G. Reuteri*, while the latter is composed of four sub-species, *G. Tetrahit, G. bifida, G. pubescens,* and *G. speciosa*. The species are self-fertile and more or less autogamous, and the degree of self-fertilisation is, on the average, inversely proportional to the flower size.

Intra-specific and inter-specific sterility in the two subgenera were studied and the various factors involved elucidated.

The original should be consulted for the detailed results, which are numerous (Müntzing, 1930).

In the sub-genus *Ladanum*, of the four species investigated, *Ladanum, angustifolia, ochroleuca* and *pyrenaica* should theoretically give six hybrids. Of these six, five were experimentally realised, while one cross, *pyrenaica* × *angustifolia* and the reciprocal cross invariably gave negative results. The cross *ochroleuca* × *pyrenaica* was only obtained with considerable difficulty, but the other crosses succeeded with greater ease. Thus the position of *G. pyrenaica* is somewhat isolated, and under natural conditions it is possible that it may only hybridise with *G. angustifolia*. *G. Ladanum*, on the contrary, forms a group with *ochroleuca* and *angustifolia* in which all three possible combinations are realised with comparative ease.

Of the five different kinds of hybrids obtained, all were partially sterile to the same extent. The exception, however, was *ochroleuca* × *pyrenaica*, in which the F_1 plant was quite as vigorous, if not more so, than the parent forms. The F_2 and F_3 generations showed great and often transgressive variation, and some of the segregates were markedly inferior in vitality. These results agree well with the fairly large morphological differences between some of the species that were crossed. *G. Ladanum* and *G. angustifolia*, however, are morphologically somewhat similar, and have been occasionally united into one species by systematists. The genetic results obtained in this investigation show that genotypically they are quite as different as the other species. The cross between them succeeded with difficulty, and the F_1 generation was luxuriant and partially sterile. Moreover, *angustifolia*, in contrast to *Ladanum*, cannot hybridise with *pyrenaica*.

From the literature on the subject and the results of this investigation, it is clear that *G. Ladanum*, and especially *G. angustifolia*, are highly polymorphic forms. Transitions also seem to occur between them in Nature, which gives the impression of an intercrossing population in which, through natural selection, two groups have arisen, *Ladanum* and *angustifolia*.

The species belonging to the sub-genus *Ladanum*, with the

exception of *pyrenaica* and *Reuteri*, have a wide geographical distribution, but with different ecological claims. Thus, *angustifolia* is a typical lime plant, whereas *Ladanum* thrives best on sandy soils, and *pyrenaica* and *ochroleuca* are mountain forms. This ecological differentiation will aid in upholding the species limits in spite of spontaneous species hybridisation, or, in other words, the whole sub-genus is a cœnospecies, comprising several ecospecies.

The chromosome number in the sub-genus *Ladanum* is $n = 8$, in the *G. Tetrahit* group, $n = 8$, for *G. pubescens* and *G. speciosa*, whereas *G. Tetrahit* and *G. bifida* are tetraploids ($n = 16$). It was found that cross-combinations *pubescens × speciosa* and *Tetrahit × bifida* gave vigorous and partially fertile hybrids, whereas crosses between the 8 and 16 chromosome forms gave embryos which soon died. Thus the sub-genus *Tetrahit* forms two natural groups, *pubescens-speciosa* and *Tetrahit-bifida*.

Under natural conditions, crosses occur within each of these groups. Hybrids between *pubescens* and *speciosa* have been described as being completely sterile; this statement is evidently incorrect, as Müntzing has found that they were partially fertile and that they gave a highly polymorphous progeny in the following generation. Exactly the same situation arises in the *Tetrahit-bifida* group, and the natural hybrids have almost escaped the attention of the taxonomist. The cause of this oversight is probably due to the polymorphism of the parent species, the slight morphological species differences, and also to the fact that the hybrids are not completely sterile, and, after self-fertilisation or back crosses, give rise to a polymorphism which must be extremely confusing and troublesome to the systematist. Here, as in other cases, the parent species are described as being very variable, from unknown causes, but in any case they are not due to species hybridisation.

In such circumstances, the comparative morphological method of distinguishing species completely breaks down and leads to false conclusions. The whole of the crossing experiments used in this work have shown that the entire range of forms in sub-genus *Tetrahit* must be referred to two, and only two, species-

cross combinations, *Tetrahit* × *bifida* and *pubescens* × *speciosa*. Under natural conditions these crosses result in two large polymorphic populations, characterised by the haploid number of chromosomes of 16 and 8 respectively. Within each group there is a clear division into separate units. Thus, the cœnospecies *pubescens-speciosa* comprises the ecospecies *pubescens* and *speciosa*, the cœnospecies *Tetrahit-bifida* comprises the ecospecies *Tetrahit* and *bifida*. The ecological conditions leading to the formation and preservation under natural conditions of these ecospecies are at present quite unknown. Müntzing gives the following diagrammatic representation of the natural grouping and the possible cross-combinations of the *Galeopsis* species :—

Cœnospecies
Ladanum (*n* = 8)

Cœnospecies
pubescens-speciosa
(*n* = 8)

Cœnospecies
Tetrahit-bifida
(*n* = 16)
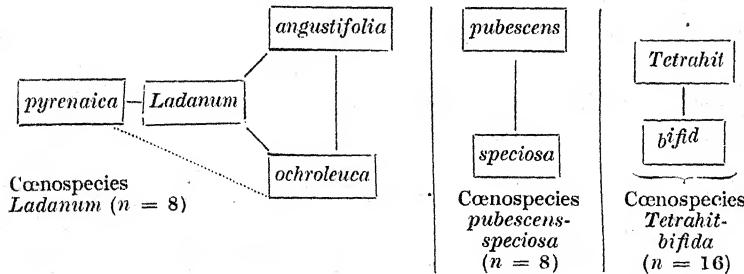
It will be seen that there are three different intersterile natural groups, the cœnospecies, *Ladanum*, *pubescens-speciosa* and *Tetrahit-bifida*. Each cœnospecies is in turn composed of ecospecies which hybridise under natural conditions, and the result is the marked polymorphism shown by the genus, but the species limitation is upheld by selective elimination.

For similar results to this work on *Galeopsis*, the papers by Clausen on *Viola tricolor* should be consulted, in which it is shown that *Viola tricolor* is a cœnospecies, which is made up of at least three ecospecies, *V. tricolor* in the strict sense, *V. arvensis* and *V. alpestris*. See also in this connection Heribert-Nilsson on the hybridisation of *Salix caprea* × *S. viminalis*.

Classificatory Units. The question arises, What are the kind of units that should be used in a system of classification? The ideal of the taxonomist is to devise a natural system of classifica-

tion, that is, a system based on natural relationships between the various forms composing the system, or, in other words, that there should be a phylogenetical foundation of the units used in such a system.

Whatever standard is used to delimit the units of a classificatory system, it will generally be agreed that these units are composed of a greater or lesser number of biotypes. In the case of plants which reproduce sexually, biotypes within such a unit are free to hybridise, and the biotypes belonging to one group may often produce fertile offspring when crossed with biotypes from another group differing widely in external morphology, and the hybrids may frequently differ very considerably in external form. The fact that the offspring of these crosses are fertile, and the relatively frequent occurrence of such hybrids in Nature, undoubtedly shows a close genetical similarity, whereas the morphological similarity is so small that they have come to represent time-honoured species. On the other hand, crosses between biotypes of close morphological similarity often give negative results. It cannot, however, be concluded on these grounds that failure to interbreed indicates no genetical relationship. The result may be due to differences in chromosome number, or to the failure of the pollen grains to grow on the foreign stigma. But on account of similarity in external morphology inter-sterile biotypes of this kind are grouped in one and the same systematic unit.

Since morphological relationship is a poor guide to phylogenetical relationship, and, further, since cytological and genetical methods are also poor in this respect, the phylogenetic grouping of biotypes becomes a difficult and complex problem. As Turesson has pointed out, natural definiteness of biotype groups does not necessarily involve common descent, and, secondly, a classification of biotypes in definite and natural groups is attainable without resort to any phylogenetical speculations. The guiding principles of this classification are based on genetical and ecological data and compose his genecological units, which have been discussed above.

It will be remembered from this and other work on the subject that the species-units, and, not least, those of Linnæan rank, of

traditional taxonomy represent compounds of very different order and magnitude. On occasion, the Linnæan species matches Turesson's cœnospecies, in other cases it corresponds to his ecospecies, and in yet others it corresponds to his agamospecies. So that it becomes difficult if not impossible to formulate one standard definition of a " species " in such circumstances.

It is the ecospecies that is of the greatest importance in this connection, and its behaviour throws much light on the origin of sub-specific units. Whether the geographical distribution be wide or restricted, the ecospecies will still consist of a greater or lesser number of biotypes. If these different biotypes were allowed to multiply in a certain area free from competition and under uniform conditions, it would follow, from all the possibilities of interbreeding, that only one kind of unit would obtain, namely, the biotypes themselves. Under natural conditions, however, there are always barriers to free intermixture, splitting up the biotype-body into still smaller units, each containing a diverse number of biotypes. Climatic barriers are of prime importance for the origin and maintenance of the group-isolations of biotypes found within the ecospecies. Corresponding to the change in climate, there is a change in the biotype characteristics of the ecospecies, and this change can only be explained by the selective influences of environmental conditions on the biotype-composition of the ecospecies in different climatic regions. Thus, owing to differences in climate, the biotype compound of the ecospecies is split into smaller groups, each specialised for a special type of climate, and these groups remain isolated, since the non-specialised biotypes which occasionally make their appearance in the respective groups would be eliminated steadily. Similarly in edaphically diversified places several distinct habitat-groups may be discovered, each characterised by definite and hereditary biotype characteristics. These climatic and edaphic units, arising and maintained by the sorting and controlling effect of climate and habitat-factors, are the ecotypes.

These ecotypes are of prime importance for a genecological system of classification, and morphological methods are altogether quite insufficient for the classification of ecospecies into ecotypes.

Ordinary taxonomical methods cannot reveal their presence, although it is of the greatest importance to recognise their presence for the understanding of the geographical distribution of the particular species.

Although analysis of genetical relationship offers the only means at present of distinguishing between hereditary variations and environmental modifications, and is therefore necessary in the study of sub-specific units, this analysis only brings us down to the ultimate units, the biotypes, but does not aid in the recognition of the grouping of biotypes in Nature. Here it is the ecological relationship between the biotypes that is the deciding factor, and, according to Turesson's dictum, " the ecotypes fulfil the requisites of sub-specific units, originating through Nature's own grouping of the biotypes."

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CHAPTER IV

FUNGI

REPRODUCTION

Introduction — Plasmodiophorales — Mycetozoa — Phycomycetes —
Chytridiales—Oomycetes—Ascomycetes.

Introduction

IT will not be possible in the space here to describe more than
a small section of the numerous problems connected with the
Fungi. The annual output of papers dealing with this important
group of plants has now reached enormous dimensions, and in
the circumstances a purely arbitrary selection of subjects and
investigations has had to be made in these pages.

It is proposed in this chapter to deal with the life-histories of
a few of the lower fungi, *e.g.*, Plasmodiophorales, Mycetozoa and
Phycomycetes, and to discuss the still debated question of whether
or not a double fusion of nuclei occurs in the Ascomycetes. The
two succeeding chapters will be devoted to the question of
heterothallism and the problem of mycorrhiza. Since 1918 a
number of important papers have appeared on the phenomenon of
heterothallism exhibited by the higher Fungi (Ascomycetes and
Basidiomycetes), especially from Canada, where Buller and his
co-workers have described a number of curious facts, and also
from the Continent, where Vandendries, Kniep and others have
contributed their quota. In this country, Dr. M. C. Rayner has
made the problem of mycorrhiza, especially in the Ericales,
peculiarly her own, and for a more detailed account of the subject
her monograph (*Mycorrhiza*) should be consulted. Mr. J.
Ramsbottom has also contributed in the elucidation of the
problem of mycorrhiza in the Orchidaceæ, while the exotrophic

mycorrhiza of forest trees has now been made a subject of extensive investigation.

Plasmodiophorales

The Plasmodiophorales form a small and isolated group among the Fungi, with no very evident relationships with other groups. They are obligate parasites on the roots of higher plants, and *Plasmodiophora brassicæ* is responsible for a large amount of damage to Cruciferous crops, and is the cause of the so-called " Finger-and-Toe " disease, " Anbury " or " Club-foot " of Crucifers.

The investigation of this group commenced with the classical work of Woronin on *Plasmodiophora brassicæ*, which extended from 1873 to 1877, and was followed by a number of other investigators. Plants attacked by *Plasmodiophora brassicæ* show swellings of irregular shape on their tap and lateral roots. These swellings increase in size, and for a time retain the same colour as the healthy roots, have a firm consistency and cut readily. As they age, the colour changes to brown, the swellings soften, and finally they become resolved into a brown, evil-smelling mass. The life-history may be briefly described as follows : in young tumours, cells are found which contain nucleated, amœboid bodies of various shapes. They are at first few in the cells, *i.e.*, in each cell, and, being more or less hyaline, are difficult to identify and distinguish from the cytoplasm of the host cell. Small amœbæ contain but few nuclei, two being the smallest number. With increase in the size of the amœbæ, there is nuclear division, which is unaccompanied by the division of the amœbæ themselves, so that these bodies become multinucleate. The nuclear division which takes place is not normal mitosis, but is generally known as " protomitosis," or occasionally as " cruciform division." [1] For an account of the reinvestigation of protomitosis in the Plasmodiophorales, the paper by Cook on *Ligniera Junci* should be consulted ; a summary of this work will also be found in the Botanical Section of *Recent Advances in Microscopy* (This Series).

[1] Horne has recently put forward the claim that ordinary mitosis occurs in these forms, and that protomitosis has been wrongly interpreted by past investigators.

In their early stages the amœbæ have the power of coalescing and forming plasmodia. This is not a process of conjugation, but merely association in a manner similar to that of the Mycetozoa (see below). With increase in size of the vegetative soma, the daughter nuclei again divide by protomitosis. The final stage in the growth of the plasmodium is marked by a change in the character of the nuclei. When the amœbæ are mature the karyosomes or nucleoli disappear, and the nuclei appear as vacuoles lying in the cytoplasm (akaryote stage). At the conclusion of the akaryote stage the nuclei become supplied with fresh stainable chromatin and proceed to divide by ordinary mitosis, and two nuclear divisions take place, distinct chromosomes being differentiated. These two divisions are usually held to constitute a meiotic phase, the first being a heterotypic and the second a homotypic division. The two nuclear divisions are followed by furrowing of the plasmodium cytoplasm, and the whole organism is divided up into a number of small fragments, each composed of a nucleus and small amount of cytoplasm. These bodies constitute the spores. The spores are at first naked, but later become surrounded by a spore wall. Decay of the host tissues releases the spores into the soil.

Up to recent years there were several gaps in the life-history of this form. In the first place, nothing was known of the fate of the spores from the time they were released from the decayed host's tissues to the reappearance of the parasite in a new host. According to Woronin, who unfortunately does not state under what conditions he made his observations, the spores give rise to a single naked body, which is drawn out to a long flagellum at its anterior end, a so-called "myxoflagellate." Secondly, the method of infection has never been satisfactorily settled. Chupp has made an examination of this point and described the presence of amœboid bodies in the root hairs, which he considered divided up to form spores. Lastly, there is the question of the position of nuclear fusion in the life-history. If the view that the last two nuclear divisions in the life-cycle constitute a meiotic phase be correct, there must have been some point at which nuclear fusion occurred. P. M. Jones investigated the life-history of this

parasite under cultural conditions, and claimed that the swarm spores released from the spores fuse in pairs to produce a zygote, which eventually formed a new plasmodium. Two kinds of gametes were produced, large and small, and Jones held that they both came from the same source.

The life-cycle of *Plasmodiophora brassicæ* has recently been reinvestigated by Cook and Schwartz. It was found that the spores germinated with difficulty, and the most satisfactory results were obtained in Knop's culture medium with the addition of 1 to 2 per cent. glucose. Swarm spores were discovered in these solutions, but their emergence from the spore was never observed. Whether the swarm spores that were, in fact, discovered had anything to do with *P. brassicæ* is a very moot point, since the authors themselves state that they were unable to obtain completely pure material of the parasite. The statement that these bodies were only found thirty-six to forty-eight hours after introduction of the infected material into the solutions, does not materially help their argument, for other and foreign spores that also happened to be present might have taken a similar time to germinate. These swarm spores were motile structures with a single apical flagellum. Similar structures were also found in slides made from very young cabbage seedlings.

Investigation of the process of infection showed the presence of small spherical bodies lying near the tips of root hairs of young seedlings. No flagellum was present, but a nucleus with a well-marked nucleolus was observed. These spherical bodies possessed the power of slow amœboid movement, and eventually travelled up the root hair. The actual penetration of the root hair was not observed, but many swarm spores were found in close association.

The young swarm spore migrated further down the root hair and the nucleus divided, and ultimately a small plasmodium was formed. A mature plasmodium might possess as many as thirty nuclei at this stage. These plasmodia grew very rapidly, and, when mature, each nucleus became surrounded by a mass of cytoplasm, and walls were laid down between them, cutting the plasmodium into a number of separate bodies. Cook and Schwartz disagree with Chupp with regard to the nature of these

bodies. It will be remembered that the latter regarded them as spores; Cook and Schwartz, on the other hand, consider them to be zoosporangia. After the separation of the plasmodium into zoosporangia, the nucleus of each zoosporangium divided to give
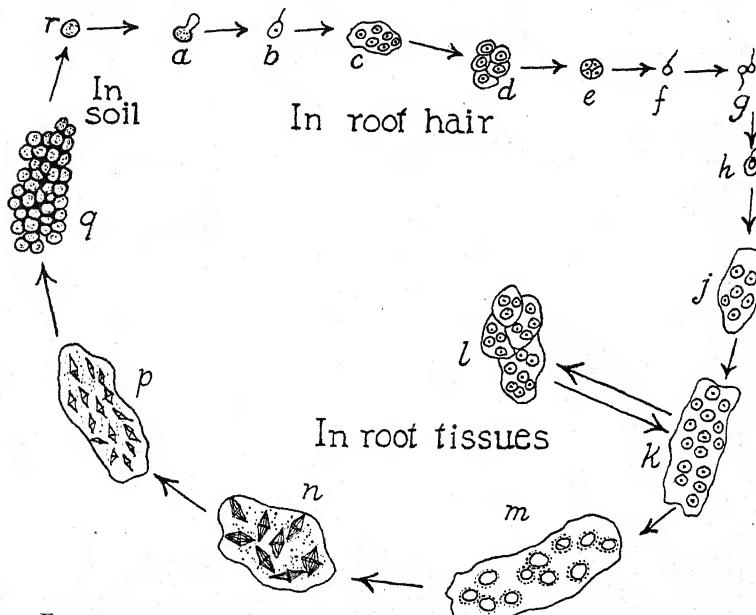
Fig. 34.—Diagrammatic illustration of the life-history of *Plasmodiophora brassicæ*. (*a*) Germinating spore; (*b*) swarm spore; (*c*) plasmodium in root hair; (*d*) zoosporangia in root hair; (*e*) zoosporangium forming zoospores; (*f*) zoospore; (*g*) conjugation of zoospores; (*h*) zygote; (*j*) young plasmodium in host tissues; (*k*) mature plasmodium; (*l*) cyst stage; (*m*) akaryote stage; (*n*) heterotypic nuclear division; (*p*) homotypic nuclear division; (*q*) spore formation; (*r*) single ripe spore. (After Cook and Schwartz, *Phil. Trans. Roy. Soc. (Lond.)*.)

four or six nuclei, each surrounded by a mass of cytoplasm. The wall of the zoosporangium now collapsed, and the zoospores made their way to the exterior. The zoospores were small, spindle-shaped bodies, with a single flagellum.

These zoospores were found to migrate from the root hairs to the epidermal and cortical layers of the root, and passed down into

the root tip. In certain cases binucleate structures were found, and the assumption was made that the zoospores can, on occasion, function as gametes and fuse in pairs. After the fusion of the zoospores to give a zygote, the latter became spherical and resembled the bodies found in the root hair. Plasmodia were now formed, as in the root hair, and the nuclei at first divided by protomitosis. Later, the akaryote stage was passed through, and this was followed by reformation of the nuclei and ordinary mitosis. Cook and Schwartz measured the average diameter of the metaphase plate in the first division of the two final mitoses in the plasmodium, and found it to be $1 \cdot 6 \ \mu$; the average of the metaphase plate in the ensuing division was found to be $0 \cdot 8 \ \mu$. It is therefore possible that these two divisions do, in fact, constitute a meiotic phase, and that the spores which are formed contain the haploid number of chromosomes.

A diagrammatic representation of the life-history of *P. brassicæ*, as described by these two investigators, is shown in Fig. 34.

Mycetozoa

The Mycetozoa, like the Plasmodiophorales, are a small and isolated group among the Fungi. In general, they are saprophytes living on dead organic matter. The most conspicuous stage is that of sporangium formation, when these appear as minute objects, some roundish in outline and about the size of small mustard seeds, while others arise in clusters of brown columns seated on black hair-like stalks. The life-histories of two species, *Reticularia Lycoperdon* and *Didymium nigripes*, have been fully investigated by Wilson and Cadman and Cadman respectively, and I am indebted to Dr. Elsie Cadman for the following summary of her own investigations as well as those carried out in conjunction with Dr. Malcolm Wilson :—

"The sporangia of *Reticularia* are found growing upon dead stumps and branches of many kinds of trees. They are particularly common on comparatively fresh stumps of pine and sycamore. The sporangium is a large lens-shaped, sessile structure, usually 4 to 6 inches in diameter. It consists of a thin, silvery skin within which is found a large mass of chocolate-coloured spores. The
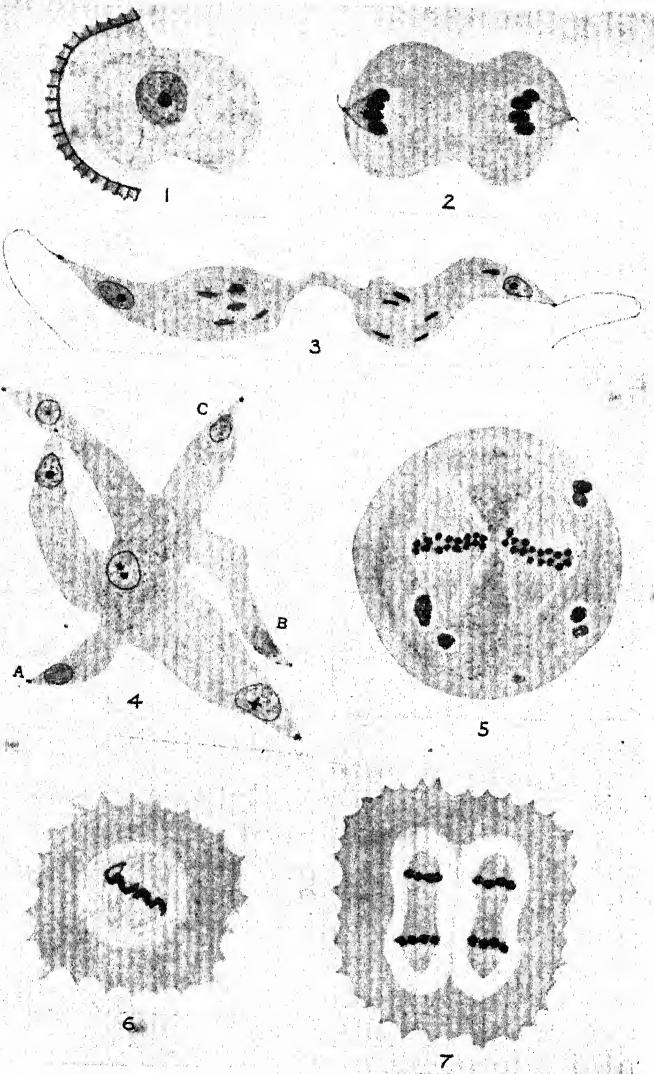
FIG. 35.—*Reticularia Lycoperdon.* (1). Ruptured spore in optical
section. (2). Telophase; the flagella is beginning to grow out
and constriction of the cell can be seen. (3). Early stage of
fusion; gametes connected by a strand. (4). A late stage of
fusion; the gametic nuclei have fused but the nucleoli are still
separate; six swarm-cells are coalescing. (5). A binucleate plas-
modium showing both nuclei in the diaster stage. (6). Metaphase.
(7). Anaphase of homotypic division. (After Wilson and Cad-
man, *Trans. Roy. Soc. Edin.*)

interior of the sporangium is divided by partitions which form a pseudo-capillitium.

" The spore is turbinate in shape, about two-thirds of the periphery being regularly rounded, and the remaining third forming a blunt cone with flattened sides ; its diameter is about 8 μ. The spore wall varies in thickness and structure in its different parts, the wall on the rounded portion being thick and strongly reticulated, whilst that on the blunt cone is thinner, without reticulation, but provided with a few short, dark-coloured spines.

" After immersion in water for fifteen to twenty minutes, the contents of the spore swell, and the coat is ruptured where it is thin and non-reticulated (Fig. 35, 1). The contents of the spore emerge as an amœboid mass of protoplasm, which gradually elongates and becomes the typical, pear-shaped swarm-cell. A deeply staining granule, the blepharoplast, is present at the pointed end, and from this a flagellum rapidly grows out, by means of which the swarm-cell swims about actively. The blepharoplast originates in the nucleus and passes out to the periphery of the cell during the transformation of the amœboid structure into the mature swarm-cell, and it maintains its connection with the nucleus by means of a striated cone-shaped structure, the flagellum-cone.

" During the next twenty-four hours the swarm-cell divides four or five times, the nucleus dividing by typical mitosis (Fig. 35, 2). Nuclear division is preceded by division of the blepharoplast, which now functions as a centrosome. The division is extra-nuclear, and 4 chromosomes are present. Apparently the swarm-cell must pass through four or five divisions before a descendant is produced which is capable of functioning as a gamete. There are no indications that male and female gametes are present ; indeed, the matings appear to be accidental. When two swarm-cells are about to fuse they approach closely and a pseudopodium is extruded from the posterior end of each (Fig. 35, 3). The pseudopodia fuse to form a narrow bridge which gradually becomes shorter and wider, thus drawing the fusing gametes closer together until cell fusion is completed. Cell fusion is followed immediately by the fusion of nuclei and nucleoli.

" Towards the completion of cell fusion, and before nuclear fusion has commenced, the pair of gametes exerts a strong power of attraction on the unpaired swarm-cells in the neighbourhood. These cluster round in large numbers and five or six of them become attached by their posterior ends to the fusing gametes (Fig. 35, 4). The protoplasm of the attached swarm-cells mingles or coalesces readily with that of the fusing gametes, and adds greatly to the bulk of the zygote formed. Meantime, fusion of the gametic nuclei has been completed and the large zygote, or plasmodium as it is now termed, is dominated by a diploid nucleus. The haploid nuclei which entered the zygote from the coalesced swarm-cells disintegrate and are digested. The plasmodium is also able to engulf and digest entire, unfused swarm-cells.

" The nucleus of the zygote is very large, and soon divides a number of times. The division is intra-nuclear, without centrosomes, and 8 chromosomes are present (Fig. 35, 5). In laboratory cultures it was never possible to obtain plasmodia which contained more than 6 or 8 nuclei, and it is very probable that the plasmodium enters the wood in which it passes most of its life at a very early stage. The length of time which the plasmodium spends within the tissues of the substratum has not been ascertained, but it is possible that it makes its entry during the spring of one year, and does not emerge to form the sporangium until the spring of the following year.

" The plasmodium emerges from the wood as a number of small masses, creamy in consistency, creamy-white in colour, and with a mulberry-like surface. These soon coalesce to produce a single, large sporangium, 2 to 6 inches in diameter. The sporangium gradually becomes smooth and rounded, loses the mulberry-like appearance, and a silvery skin begins to form. Within the sporangium the creamy material darkens and finally gives rise to chocolate-brown spores.

" During the progress of the external changes described above, internal changes also take place. Much of the protoplasm present degenerates to form the wall and pseudo-capillitium of the sporangium. In the protoplasm which does not degenerate, the nuclei undergo typical heterotypic and homotypic divisions, which

are intra-nuclear with four chromosomes, and no centrosomes are present (Fig. 35, 2). Following upon meiosis, the protoplasm is cleaved into successively small portions until uninucleate spores have been formed. The spore-wall is laid down in two layers, an extine and an intine, and during its formation considerable activity is displayed by the nucleus and protoplasm of the spore. The extine is provided with an additional layer of thickening over two-thirds of its surface, which is laid down in the form of peg-like masses of colourless material. The reticulation of the surface over this area is due to the contraction of the spore as a whole during the ripening process, which causes the outer layer of the extine to fall into folds. The spines which are present on the remaining one-third of the surface of the spore are also due to its contraction during ripening.

" The sporangium in *Didymium nigripes* consists of a spherical head and a massive stalk. The head of the sporangium is thickly incrusted with a layer of stellate crystals of calcium carbonate. The stalk is dark-brown or black in colour, and tapers from a broad base to a point at its junction with the head. The sporangia are frequently found on decaying vegetable material, particularly on dead leaves.

" The spore is spherical in shape, dark-brown in colour, and regularly studded with stout spines over its entire surface. The spore coat is one layer thick and there is no definite thin portion, so that it differs markedly from that of *Reticularia*. The contents of the spore swell rapidly after immersion in water or a nutrient solution, and rupture the spore-coat in about thirty minutes. The protoplast then contracts and nuclear division followed by cell division, takes place, resulting in the formation of two swarm-cells which emerge through the fissure in the spore-coat, with a small flagellum already present, about one and a half hours after the spores were immersed in the liquid (Fig. 36, 1).

" The mature swarm-cell is not so active as that of *Reticularia*, and spends much of its time in the amœboid condition. During the twenty-four to thirty-six hours after its emergence it divides three or four times, the divisions being extra-nuclear with 4 chromosomes, and its descendants then become transformed into
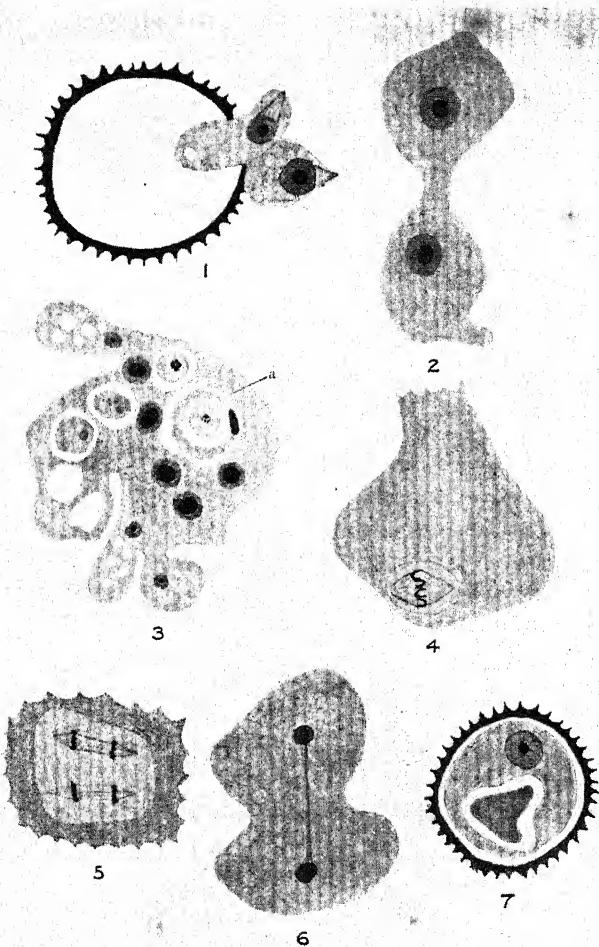
FIG. 36.—*Didymium nigripes* var. *xanthopus.* (1). Emergence
of the swarm-cells. (2). Stage in the contraction of the bridge
between the fusing gametes. (3). Plasmodium which has
recently coalesced with three uninucleate plasmodia. Five of the
six large nuclei are shown. The three small nuclei belong to the
uninucleate plasmodia which have coalesced. At (*a*) a large
uninucleate plasmodium has been engulfed which has itself
engulfed a myxœba. (4). Metaphase. (5). Anaphase. (6).
Late telophase. (7). A spore showing the presence of a single
vacuole containing a yellowish body. There is a space between
the protoplast and the spore-wall. The nucleus is normal and
the protoplasm stains less deeply. (After Cadman, *Trans. Roy.
Soc. Edin.*)

myxamœbæ in which flagella, blepharoplasts and flagellum-cones have disappeared. The myxamœbæ function as gametes and fuse in pairs, as in the case of the swarm-cells in *Reticularia* (Fig. 36, 2). No coalescence of unfused myxamœbæ, or of swarm-cells, with the fusing gametes has been observed.

"The uninucleate plasmodium does not increase in size by nuclear division, as it did in *Reticularia*. Coalescence of small plasmodia with those which are larger takes place. The uninucleate plasmodia do not develop uniformly. Those which are first formed probably develop more rapidly than those which are younger, and are in a condition earlier to ingest unfused myxamœbæ. Therefore the older plasmodia soon become distinctly larger and apparently form centres of attraction for smaller plasmodia which contain no inclusions. The latter cluster round the former in large numbers and coalescence takes place (Fig. 36, 3). Since all the coalescing structures are diploid, the nuclei which enter the central plasmodium are not digested, but increase in size and become useful nuclei of the large plasmodium. The latter, therefore, is enabled to increase the number of its nuclei very rapidly without the process of mitosis. The larger the plasmodium the greater its power of attraction for those which are smaller, and the final result is usually a single large plasmodium with which all those which were smaller have coalesced.

"It is almost certain that the process described above is the means by which the plasmodium attains its adult size in nature, and this probably accounts for the fact that nuclear divisions in the plasmodium are difficult to secure. It is only occasionally in very large plasmodia or in plasmodia which are somewhat isolated and have no opportunity of coalescence that divisions may be obtained. The divisions resemble those in the plasmodium of *Reticularia*, for they have 8 chromosomes, they are intra-nuclear, and centrosomes are absent.

"When the mature plasmodium is about to form sporangia, the protoplasm becomes heaped up into small, irregular masses which rapidly become globular in shape. The rounded masses of protoplasm are quite white in colour, because all impurities

have been left behind on the surface of the nutrient medium.
The massive stalk is gradually built up from degenerating proto-
plasm; the sporogenous tract being confined to a very narrow
layer on the periphery of the globular head. The capillitium and
wall of the sporangium are laid down very early and before any
development of the sporogenous protoplasm has taken place.
The threads of the capillitium are laid down on the walls of furrows
which pass inwards from the surface, and outwards from the
columella in the centre of the columella. The walls of these
threads and the walls on the surface of the sporangium and
columella are probably formed as a result of a chemico-physical
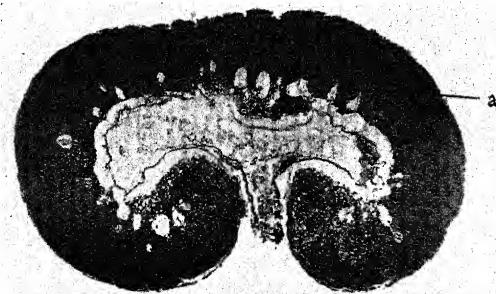
FIG. 37.—*Didymium nigripes* var. *xanthopus*. A section through
a young sporangium at noon. (*a*) Furrow containing capillitial
thread. (After Cadman, *Trans. Roy. Soc. Edin.*)

reaction taking place on the surface of protoplasm exposed to
air and controlled by the protoplasm itself (Fig. 37).

"Meiosis and cleavage of the sporogenous protoplasm occur
simultaneously. Meiosis, as in *Reticularia*, consists of typical
heterotype and homotype divisions. These divisions are intra-
nuclear, 4 U-shaped chromosomes being present in the heterotypic
division and 4 spherical chromosomes in the homotypic division.
Centrosomes, however, are absent. The final cleavage of the
sporogenous protoplasm to form single spores takes place during
the concluding stages in the homotypic division (Fig. 36, 4).

"The spore-wall consisting of a single layer is deposited in
the same manner as in *Reticularia*. After its deposition a certain

amount of material is left behind in the form of a crystalline mass which is gradually absorbed by the developing spore (Fig. 36, 5). As the spore ripens the protoplasm within contracts and the spore wall falls into wrinkles which form the spines, which are to be found scattered over the surface of the mature spore.

" *Didymium nigripes* is most probably a more advanced member of the Mycetozoa than *Reticularia Lycoperdon*. Evidence for this view is to be found in the presence of myxamœbæ, fusion of amœboid, in place of flagellated gametes, the coalescence of plasmodia instead of swarm-cells, and the formation of a true instead of a false capillitium."

Phycomycetes

The life-histories of the Phycomycetes have been dealt with so adequately by two recent text-books on the subject—Gäumann (*Vergleichende Morphologie der Pilze*) and Gwynne-Vaughan and Barnes (*Structure and Development of the Fungi*)—that only a few forms need be considered here. There has been a revival of interest in the study of the life-histories of the Chytridiales, and, since the publication of the two books mentioned above, a few papers have appeared describing life-histories of these curious and interesting fungi. With regard to the higher Phycomycetes (Oomycetes), Kanouse has published two memoirs on the water moulds, and considers that the Saprolegniales should suffer further sub-division (see below).

Chytridiales. Bayley Butler and Buckley, and also E. J. Butler, have described the life-cycle and morphology of a Chytrid parasiting the ova of *Fasciola hepatica*, the liver fluke of sheep.

The parasite belongs to the genus *Catenaria*, which was established in 1876 by Sorokin for a Chytridiacean fungus attacking eelworms. A decade later (1885–86) Dangeard carried out a detailed investigation of the fungus, and claimed that it was also present in the thalli of *Nitella*. Up to the time of these two present investigations the known hosts of *Catenaria* were nematode worms, rotifer eggs and the cysts of infusoria.

The form to be described now (*C. anguillulæ*) was discovered in the ova of the liver fluke of sheep, and an investigation of the
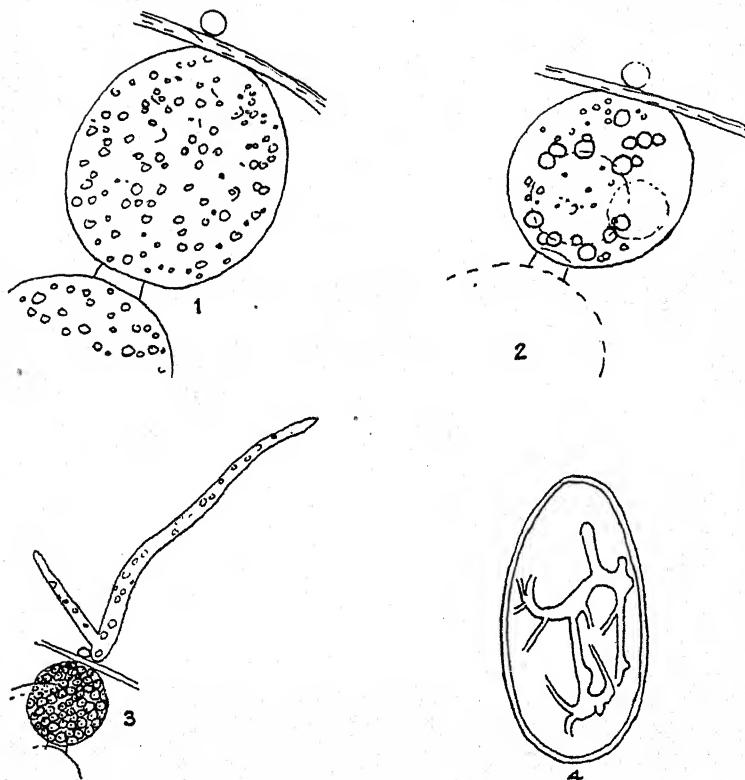
FIG. 38.—(1). Sporangium with small and evenly distributed granules. (2). Contents of sporangium now becoming vacuolate. Outline of a second sporangium developed from the same thallus can be seen. The narrow portion of the thallus between the two sporangia, the isthmus, is empty. (3). Zoospore formation complete. (4). Earliest stage of thallus formation. A main recurved axis formed by a rather stout hypha with irregular swellings and with a single blunt branch at the top can be seen. All the other branches are rhizoids with exceedingly thin walls. No septa are visible at this stage. ((1). (2). (3). After Bayley Butler and Buckley, *Sci. Proc. Roy. Dubl. Soc.* ; (4). after E. J. Butler, *Anns. Bot.*)

life-history and method of entry into the ova has been conducted by Bayley Butler and Buckley, while E. J. Butler has carried out a morphological examination of the fungus in the egg.

The zoospores are uniciliate, and more or less oval for the major part of their free-swimming life, but towards the close of this period they become spherical, and amœboid movement becomes apparent in some degree. In swimming, the cilium is carried in the posterior position. The free-swimming period lasts from fifteen minutes to seven hours, and when infection of the eggs is about to take place the activity of the zoospores becomes considerably diminished. Infection may not always take place immediately; the zoospore approaches the egg for a second or so, and then swims away, and in some cases does not make a further attempt at infection and disintegrates. When, however, infection does take place, the zoospore comes to rest on the surface of the egg, and can be seen to be slightly flattened at the point of contact. A membrane is now formed round the zoospore, and this remains firmly adhering to the ova after entry has been effected. The contents of the zoospore enter the egg through a small perforation and form a cyst within, which is attached to the shell at the point of entry; this is the first sporangium. A fine mycelia outgrowth is now sent out and forms a sporangial swelling, a slight " isthmus " separating the first sporangium from the second (Fig. 38). Cross walls are laid down between the two sporangia and the separating isthmus contracts considerably. A beak is now put out at the base of the first sporangium, which is rhizoidal in nature. A number of granules can be seen within the sporangium, which later become concentrated into definite groups—the developing zoospores. The size and number of sporangia vary very considerably; six to twelve is a very usual number in each egg, while the diameter of the sporangia may vary between $50\,\mu$ and $15\,\mu$; there is also much variation in shape. Discharge of the zoospores takes place through a beak. Prior to discharge there is much activity among the zoospores within a sporangium, rupture of the apex of the beak now takes place by the " blowing out " of a transparent hemispherical cap which covers the tip, and eventually the zoospores are discharged.

The morphology of the thallus shows it to consist of a swollen main axis with a tendency to branch, and from this a number of thin-walled rhizoids grow out (Fig. 38). At a later stage the

Oomycetes. Kanouse has made a study of the Saprolegniales and considers that the order should now be subdivided into two further orders, Blastocladiales and Leptomitales. She has also discovered the presence of an antheridium in *Blastocladia globosa*; a hitherto unrecorded fact. The antheridia arise from the wall of the main stalk or its lobes. They are by no means common, and the process of fertilisation was not observed. In the Fungi the sex act usually takes place by the passage of the contents of the male gametangium into the female organ. The exception to this rule is *Monoblepharis*, in which motile sperms with a single cilium are formed. From the fact that the antheridial branches in *B. globosa* extend beyond the basal cell bearing the sessile oogonium, Kanouse is of the opinion that motile sperms are involved in the sex act here.

Zoospore formation in *Blastocladia* is also peculiar. In *B. Pringsheimii* the formation of zoospores differs materially from *Saprolegnia*. The protoplasm at first becomes coarsely granular, and then finally granular and very refractive. Vacuoles soon make their appearance near the sporangial wall, and the number of vacuoles approximately corresponds to the number of zoospores eventually formed. These vacuoles shift their position quickly, coalesce, and cause the protoplasm to take a central position in the sporangium. The protoplasm now shows a wavy margin, and the vacuoles continue to cut into this, leaving large, irregularly shaped masses. The protoplasm finally expands and fills the sporangium. During the early stages of zoospore formation, while the vacuoles are rapidly dividing the protoplasm into small units, an exit-papilla makes its appearance, and this at first looks like a small plug extending downwards into the sporangium from the apex, but before the sporangium opens the exit-papilla becomes large and conspicuous.

From the fact that the zoospores are monoplanetic in the Leptomitaceæ, and diplanetic in the Saprolegniaceæ, Kano... considers it more correct to remove this family from the Sap... Leptomitaceæ, and to place it in a separate order, Leptomitales, wi... one family Leptomitaceæ. Further evidence for this separatio... is the segmentation of the hyphæ, the presence of only a singl...

FIG. 39.—(1) and (2). *Entophlyctis heliomorpha.* (1). Mature sporangium with escaping zoospores. (2). Mature resting spore with a single branched rhizoid. The trunk has undergone cleavage. (3) and (4). *Diplophlyctis intestina.* (3). A late stage in the development of the sporangium. Two sporangial necks have begun to form and the apophysis is comparatively small. (4). A mature sporangium with escaping zoospores. (After Karling, *Amer. J. Bot.*)

point of origin of the rhizoids and the thallus. At first the ...rangium, apophysis, and rhizoids are unicellular, but at a later ...le the sporangium is delimited from the apophysis by a ...nsverse wall. As in the case of *Entophlyctis heliomorpha,* *Diplophlyctis intestina* also forms resting spores.
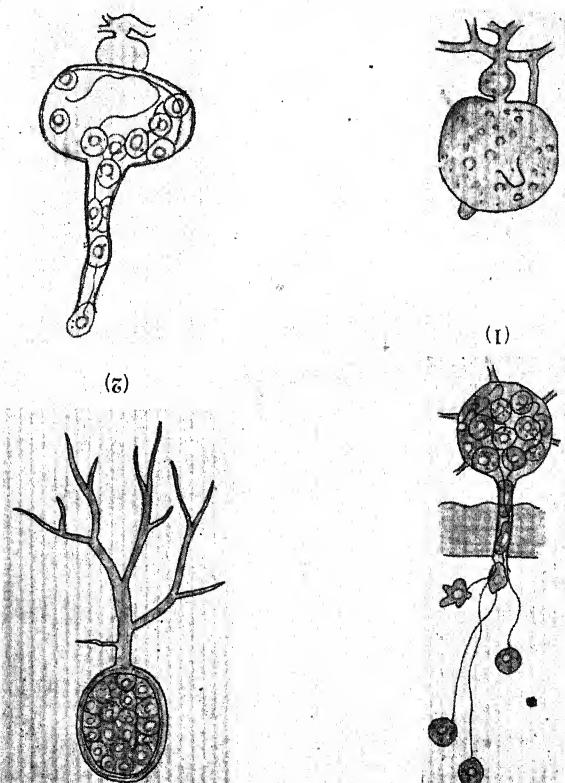
contents of this mycelium retract into the swollen parts, which are then cut off by septa and become sporangia.

Schwartz and Cook have described the life-history of a new species of *Olpidium* (*O. radicale*) which infects the roots of *Veronica Beccabunga*. The zoospores here are uniciliate and pear-shaped. Entry into the host is effected through a root hair. When entry about to take place the cilium is withdrawn and an infection tube is put out. The contents of the zoospore pass through this tube into the host, and on occasion it may, when once entry has been effected, develop a fresh cilium and become active within the host cell. A sporangium is formed within the root hair, which is at first uninucleate, but later becomes multinucleate, the nuclear divisions being mitotic in character, and quite distinct chromosomes can be made out on the achromatic figure. During its whole period of development the sporangium is surrounded by a cellulose wall. If two sporangia be present in the host cell, they frequently increase in size without nuclear division, the smaller approaches the larger, and a beak-like process is put out which comes into contact with the wall of the larger sporangium. Fusion of the contents was not observed, but was assumed to take place, since the contents of the smaller was found to be empty, while the larger now contained two nuclei. Presumably the smaller sporangium is playing the part of an antheridium, while the larger is functioning as an oogonium. The two nuclei fuse, and a thick wall is formed round the oospore. A number of mitotic divisions take place, and a considerable amount of chromatin is extruded into the cytoplasm. The contents of the oospore now divide up to form zoospores. This sexual process has apparently nothing to do with the over-wintering of the fungus, since oospores were found at all times of the year. Asexual sporangia are also formed, and these give rise to zoospores directly. Discharge of the zoospores takes place through an exit tube.

and in members of the Rhizidiaceæ have been investigated by this fus. *Entophlyctis heliomorpha* and *Diplophlyctis intestina*. septation species are weak parasites or saprophytes on the contained t. *Entophlyctis heliomorpha* is a Chytrid of wide cell develop in the dead nodal, internodal and cortical cells of the

The species, however, which has led to wide divergence of interpretation of the behaviour of the sexual nuclei is the Discomycete *Pyronema confluens*. The cytology of this form was first investigated in 1900 by Harper. *Pyronema confluens* is to be found on burnt ground, and occasionally on the remains of decayed leaves or leaves that have been charred. The fructifications possess a characteristic pink colour and the sexual apparatus consists of multinucleate antheridium and multinucleate oogonium and trichogyne. At the time of fertilisation the tip of the trichogyne comes into contact with the tip, or more frequently the side of the antheridium. The walls of the antheridium and trichogyne now break down at the point of contact and a pore is formed. The nuclei present in the trichogyne degenerate, and the male nuclei migrate through this passage into the oogonium. The wall at the base of the trichogyne also breaks down, and the male nuclei travel through and mingle with the female nuclei in the oogonium. After the migration of the male nuclei from the antheridium is completed, a fresh wall is laid down across the base of the trichogyne. According to Harper, male and female nuclei now pair and then fuse (Fig. 40, 1). Ascogenous hyphæ are budded out. The nuclei pass into these and the hyphæ elongate, branch freely, and undergo septation, and at last bend over and give rise to asci from their penultimate cells which contain two nuclei, and again, according to Harper, a second nuclear fusion takes place here. The penultimate cell now forms the ascus, three nuclear divisions follow and the eight nuclei of the ascospores are formed.

This interpretation of a double nuclear fusion was followed by later investigators for other Ascomycetes, *e.g.*, Blackman and Fraser for *Humaria granulata*, in which only an oogonium is present, and the female nuclei were described as fusing in pairs, and *Humaria rutilans*, in which neither oogonium nor antheridium are present, and here it was claimed that the vegetative nuclei first fused in pairs (Fraser).

It is clear that if two nuclear fusions occur in the life-cycle of these forms, there must be two reduction divisions to compensate for the quadrupling of the chromosome numbers. After fusion, three nuclear divisions follow; the first is heterotypic

and the second homotypic, and these together constitute a meiotic phase; a third division now follows, and it is the interpretation
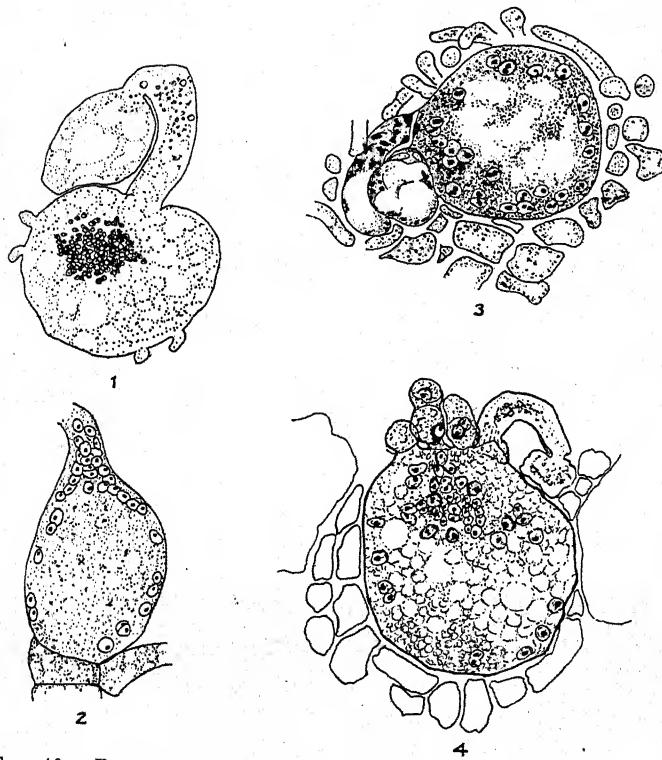
FIG. 40.—*Pyronema confluens*. (1). Oogonium showing fusion of male and female nuclei. (2). Oogonium showing association of male and female nuclei in pairs. (3). Oogonium showing fusion of male and female nuclei. (4). *P. domesticum*. Oogonium after entry of male nuclei. ((1). After Harper, *Anns. Bot.* (2). After Claussen, *Zeit. f. bot.* (3). After Gwynne-Vaughan and Williamson, *Anns. Bot.* (4). After Tandy, *Anns. Bot.*)

of the nature of this division that has led to ... especially if he is logical controversy in the group. In 190... subject. It is clear Fraser that the third division in the a... the Claussen and the division, bearing the same relation to... ve opinions with a good

meiosis bears to the fusion of the sexual nuclei. For this reduction division the term *brachymeiosis* was proposed, and was first recorded by her for *Humaria rutilans*. The number of chromosomes in the nuclei of the ascogenous hyphæ, in the first and second divisions in the ascus, and in the prophase of the third division, were sixteen, but in the telophase of the third division eight were recorded.

The presence of a brachymeiotic reduction was described in several other Ascomycetes, *e.g.*, by Carruthers in *Helvella crispa*, and by Fraser for *Lachnea stercorea*. In the latter, four chromosomes are described for the first and second divisions in the ascus and two in the telophase of the third division.

In 1912, however, Claussen, in a particularly able paper on *Pyronema confluens*, brought forward most cogent evidence to show that there was only a single nuclear fusion in the life-history (that in the ascus), and such being the case, the need for a second reduction division vanished.

In essentials the life-history of *P. confluens*, as described by Claussen, was similar to that given by Harper, but Claussen failed to find fusion of the male and female nuclei in the oogonium. On the other hand, he demonstrated that the nuclei first paired in preparation for their ultimate fusion in the ascus, and he claimed that the members of each pair were respectively male and female. The haploid number of chromosomes was found to be twelve, and there was no change in number in the third division in the ascus. Claussen, therefore, held that there was only one fusion in the life-history (that in the ascus), and only a single reduction (meiosis). The chief points upon which Claussen relied for his views were: (*a*) the presence of paired nuclei in the ascogenous hyphæ; (*b*) the failure to observe fusions in the oogonium, or, if such were found, that they were merely pathological phenomena; and (*c*) the discovery of as many chromosomes in the third division first nucleus pairs the first (Fig. 40, 2).

It is clear that if but exception, the life-cycles of Ascomycetes these forms, there must after Claussen's investigation followed the pseudo the quadrupling of the that there was only a single fusion, that apex to scus, three nuclear div of the alleged presence of a second

reduction or brachymeiotic division fell upon somewhat evil days.

Recently, however, there has been a good deal of revival in the view that two fusions actually do exist in the life-history of these forms. It is not necessary here to deal in detail with the various papers that have appeared supporting Claussen's views. For example, S. Jones has investigated the nuclear life-history of the parasite *Rhytisma Acerinum* which infects various species of *Acer* (maple and sycamore). The archicarps are described here as arising as lateral structures, and several are found in each apothecium. A branch destined to become an archicarp may consist of from two to five cells, one of which is always conspicuously larger than the rest. This large cell is the oogonium, and no antheridium was found to be present. Jones failed to discover any fusions of the female nuclei in the oogonium, but the nuclei paired and passed into the ascogenous hyphæ, and the only fusion that could be discovered in the life-history was that in the ascus. Chromosome counts in the three divisions in the ascus showed that the chromosome number (five) was the same throughout in all three divisions and brachymeiosis was absent.

Bagchee has investigated the divisions in the ascus of *Pustularia bolarioides* and also failed to find a brachymeiotic division. The diploid number of chromosomes in this species is 32, and 16 bivalents made their appearance at the prophase of the first division. In the next two divisions 16 chromosomes could be counted, *i.e.*, the third division was purely vegetative and not brachymeiotic.

It is difficult in work of this nature to sift the prejudiced from the unprejudiced investigation. The interpretation of a series of slides in any cytological investigation necessarily presents difficulties, and it is a simple matter for an investigator to observe certain things that he is anxious to observe, especially if he is suffering from preconceived ideas about the subject. It is clear from a perusal of the literature that both the Claussen and the brachymeiotic school hold their respective opinions with a good deal of tenacity of purpose.

An apparently impartial investigation, however, is that of Elliott on *Ceratostomella fimbriata*, which is the cause of the so-called "black rot" of sweet potatoes. *C. fimbriata* is a member of the Sphæriales, and is possessed of a uninucleate antheridium and uninucleate oogonium. Elliott recorded the passage of the male nucleus into the oogonium and its subsequent fusion with the female nucleus. A mistake or misinterpretation in this case appears to be almost impossible, and Elliott himself appears to have been somewhat surprised at finding this fusion in the oogonium, but he is particularly emphatic on the point.

Perhaps the most curious nuclear life-cycle that has up to the present been described for the Ascomycetes is by Tandy for *Pyronema domesticum*. *P. domesticum* is often to be found on damp wallpaper, and is very closely related to *P. confluens*. The sexual organs, oogonium and antheridium, are multinucleate structures, and formed in the same manner as in *P. confluens*. The passage of the male nuclei into the oogonium is a fairly protracted affair. Following upon the entrance of the male nuclei into the oogonium, there is a dense massing of nuclei towards the upper region of the oogonium, and Tandy describes the fusion of nuclei in pairs at the fringe of this mass (Fig. 40, 4). Ascogenous hyphæ now bud out from the oogonium, and when the tips of the ascogenous hyphæ bend over to initiate the young asci, simultaneous division of the two nuclei that are present takes place, and shows an interesting deviation from the course indicated for other Ascomycetes by previous observers. Some of the nuclei were found to show 7 chromosomes on the spindle at metaphase and 14 (7 to either pole) in anaphase, and accordingly are in the haploid condition; in others, however, 14 were found at metaphase and 20 could be counted on their way to the poles, *i.e.*, the diploid number. Fusion in the ascus was observed, and in the meiotic divisions of the definitive nucleus it was sometimes observed to be diploid and sometimes tetraploid (Fig. 41). Tandy interprets his result as follows: that the male and female nuclei in the oogonium fuse on occasion, and when this occurs the third division is brachymeiotic in nature; when the nuclei fail to fuse in the oogonium

and the only fusion in the life-cycle is in the ascus, the third division is purely equational. Tandy puts forward the sugges tion that *P. domesticum* is a transitional form among the Ascomycetes.

Gwynne-Vaughan and Williamson (1930) have recorded a
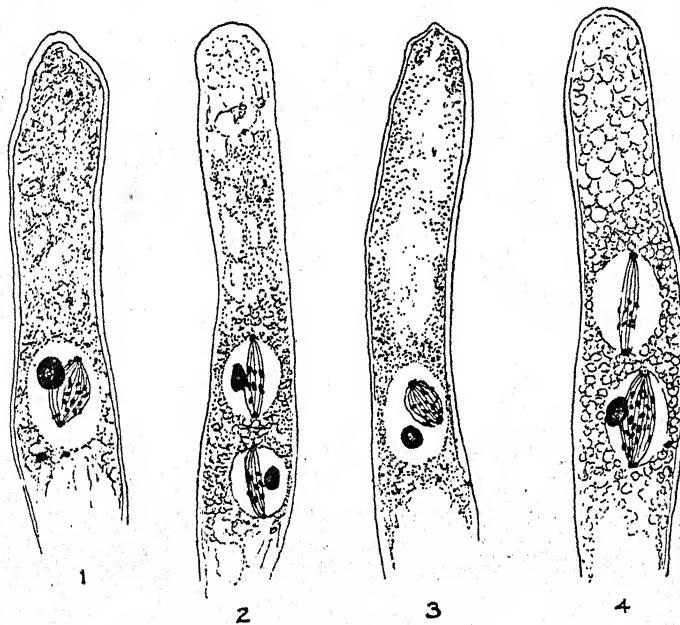
Fig. 41.—*Pyronema domesticum.* (1). Anaphase of first division in the ascus (diploid nucleus). (2). Metaphase of second division in the ascus (haploid nucleus). (3). First division in the ascus (tetraploid nucleus). (4). Anaphase of second division in the ascus (diploid nucleus). (After Tandy, *Anns. Bot.*)

brachymeiotic reduction in the heterothallic species *Humaria granulata.* They have confirmed the earlier work of Blackman and Fraser on this form, and find that in the absence of an antheridium the nuclei of the oogonium fuse in pairs. The definitive nucleus of the ascus shows 8 gemini at the first division, and 8 chromosomes pass to either pole of the spindle. In the second and third divisions 4 chromatin masses were

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found. Apparently 4 is the haploid chromosome number here.

Gwynne-Vaughan and Williamson (1931) have also reinvestigated the life-history of *Pyronema confluens*. From chromosome counts in the spores the haploid number was found to be 6 and not 12. The formation of the sexual apparatus is described, and merely confirms in essentials the descriptions of previous observers. When the male nuclei entered the oogonium the female elements were found to arrange themselves around the walls of the oogonium or were grouped together, especially near the base of the trichogyne. After entrance of the male nuclei, the nuclei in the oogonium could be seen to lie in pairs, and fusion soon followed, and all fusions in a given oogonium were found to take place at about the same time (Fig. 40, 3). Ascogenous hyphæ now appeared, and the nuclei flowed into them in an almost continuous stream. There was at the same time no suggestion of a paired arrangement of the nuclei, odd and even numbers in the groups of nuclei being equally common. Moreover, the ascogenous hyphæ were usually narrow at the point at which they leave the oogonium, and only allowed of the passage of one nucleus at a time. The free portions of the ascogenous hyphæ were swollen, and soon gave rise to one or more relatively narrow branches in which the nuclei lay in single file. Mitosis was found to take place at least once simultaneously in all the nuclei of a group of ascogenous hyphæ, and also in nuclei still in the oogonium. A curious feature that was ascertained was the fact that all the critical stages, such as mitosis in the spore, passage of the male nuclei into the oogonium, the fusion of male and female nuclei, mitosis in the oogonium and ascogenous hyphæ may be sought in vain, but in material in which they are found to occur they are of frequent occurrence, numbers of cells or organs being at the same stage of development. It would appear that development of a cell or organ is delayed till it receives some external stimulus, and the suggestion is put forward that this necessary stimulus may be sunlight.

Counts of the number of nuclei in the oogonium before the entry of the male elements, after entry, and oogonium with ascogenous hyphæ were made and are recorded in Table V.

Table V

	Number of oogonia.	Smallest number of nuclei.	Largest number of nuclei.	Average number of nuclei.
1. Oogonium before entry of male nuclei	18	98	201	150
2. Oogonium with fusing nuclei	18	182	393	276
3. Oogonia with ascogenous hyphæ	18	96	211	163
4. Difference between (1) and (2)	—	84	192	126

These figures record an increase in the number of nuclei in the oogonium at the time of fertilisation and subsequent decrease to about the same figure, and are considered to furnish evidence of a fusion in the oogonium.

Both in the ascogenous hyphæ and also in the croziers it is possible to count the number of chromosomes. Twelve were found in the prophase of division (*cf.* Claussen) and a considerably larger number in the anaphase, and in favourable cases 24 could be recognised, 12 to either pole (Fig. 42). Since 6 were found in the germinating spore, 12 is the diploid number, and indicates that fusion has taken place in the oogonium. After fusion of the two nuclei in the penultimate cell of the crozier to give the definitive nucleus of the ascus, the tetraploid condition is obtained. At the beginning of the formation of the spindle in the first division in the ascus, 12 gemini could be counted, and at the close of the heterotypic division 12 chromosomes were found to pass to each pole. At the metaphase of the homotypic division (second division in the ascus), 6 chromosomes could be counted, and 6 were also discovered to be present in the third division. Since 12 gemini were present in the meiotic prophase, this number represents 24 chromosomes. At anaphase 12 chromosomes passed to either pole, and the daughter nuclei were therefore in the diploid condition. Finally, a second reduction is achieved in the remaining divisions in the ascus, and the number is reduced to 6 and the haploid condition is restored.

It will be remembered that Tandy put forward the suggestion that *Pyronema domesticum* was a transitional form among the Ascomycetes, and by this means attempted to reach a compromise

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between the school that holds that two fusions occur in the life-history and the school that holds there is but a single fusion. This latest paper by Gwynne-Vaughan and Williamson places the matter in the melting-pot once more. Claussen made the
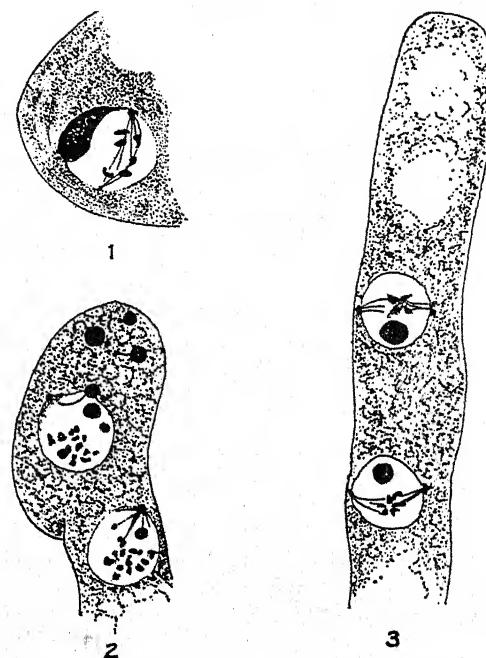
Fig. 42.—*Pyronema confluens.* (1). Prophase in one of the nuclei of the germinating spore, showing six chromosomes, the haploid number spread along the spindle. (2). Prophase in the two nuclei of a crozier ; each shows twelve chromosomes. (3). Metaphase of the second division in the ascus, showing six chromosomes. (After Gwynne-Vaughan and Williamson, *Anns. Bot.*)

categorical statement, " Ein Brachymeiosis existiert nicht," and claimed that the haploid number of chromosomes in *Pyronema confluens* was 12, but as Tandy has pointed out, in no case does he show 12 chromosomes going to either pole, and, in fact, in one figure he shows 4 passing to one pole and 5 to the other. Gwynne-Vaughan and Williamson have counted the number of chromo-

somes in the spores and state the haploid number to be 6, and it seems scarcely within the realms of possibility that such competent workers could have made a mistake. Again, although mis-interpretation is possible in multinucleate forms such as *Pyronema*, such a situation is difficult to imagine in forms with uninucleate sexual organs, *e.g.*, *Sphærotheca Humuli* and *Ceratostomella fimbriata*, in which the problem is reduced to its simplest propor-tions. As far as can be seen at present, on the available evidence, in some at least of the Ascomycetes there are two nuclear fusions in the life-cycle, and reduction in the chromosome numbers is brought about in two stages.

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CHAPTER V

FUNGI (*continued*)

HETEROTHALLISM

Heterothallism in the Zygomycetes and Oomycetes—Ascomycetes—Basidiomycetes.

Zygomycetes and Oomycetes. In 1904 it was shown by Blakeslee that zygospore formation in certain of the Mucorales was dependent upon hyphæ of two different strains coming into contact. In the absence of the opposite strain asexual reproduction continued indefinitely. Since the two strains concerned were morphologically similar, they were termed by Blakeslee " plus " and " minus." Although morphologically similar, the plus and minus strains are physiologically different, and it is only when these two strains with their dissimilar physiological constitutions come into contact that sexual reproduction takes place.

Those species of the Mucorales in which the presence of two strains is necessary for zygospore development were termed by Blakeslee " heterothallic," in contradistinction to the so-called " homothallic " forms, in which no such physiological differentiation exists. Prior to this work a number of theories had been advanced to account for the somewhat erratic appearance of the sexual organs in the Mucorales. Such factors as respiration, the nature of the medium, humidity of the atmosphere, and a number of other causes had been invoked to explain the production of zygospores in the order.

More recently Blakeslee and his co-workers have attempted to extend further, along biochemical lines, the physiological differences that must exist between these two strains or sexes. The results that have been obtained up to the present cannot, however, be said to be

of a very encouraging nature. According to Satina and Blakeslee (1925, 1926), who used the " Gosio Reaction," which involves the capacity of the living cell to absorb and reduce salts of tellurium and selenium to their respective elements (black in the former case and red in the latter), the (+) strains of *Absidia Blakesleana* and *Circinella spinosa*, grown on a 2 per cent. agar medium of malt and dextrose and 0·1 per cent. peptone, show on the average greater powers of reduction than the corresponding (−) strains. In actual practice it was found that the best results were obtained with either sodium or potassium tellurite, rather than with the corresponding selenium salts, as the red colouration produced in the second case did not stand out so well as the black of the reduced tellurium. Similarly, with Manolov's reagent (reduction of potassium permanganate), the (+) strains of *Mucor* (sp.), *Phycomyces nitens*, *Rhizopus nigricans*, *Syncephalastrum*, *Absidia* and *Parasitella simplex*, on the average again showed a greater power of reduction than the (−) forms. In this respect the (+) strains of the Mucorales behave like the female sex of the higher animals and dioecious green plants, and the (−) like the male sex.

Satina and Blakeslee have made some interesting observations on *Parasitella*. *Parasitella* is a parasite on other members of the Mucorales. It was suggested by Burgeff that the parasitism exhibited here had developed by way of an imperfect sexual reaction. Satina and Blakeslee have in large part confirmed this suggestion. Burgeff, for example, found that with species of *Rhizopus* both the plus and minus strains of *Parasitella* formed galls with either strain of the host, whereas that from which he termed the plus strain of *Parasitella* only formed galls with the minus strains of *Absidia glauca* and *A. cærulea*, and the minus race or strain of *Parasitella* with the plus race or strain of *Absidia*. Satina and Blakeslee were able to confirm the curious cytological phenomena associated with gall formation, in which the cell contents of host and parasite become mingled with the simultaneous formation of the gall. Subsequently outgrowths arise from the base of the gall and are subtended by a thick-walled storage cell. Unlike Burgeff, they were able to ascertain that there was communication between gall and storage cell.

It has now been shown by Blakeslee and his co-workers that the heterothallic Mucors are strictly dimorphic, and that there is no evidence of sex intergrades. The suggestion is put forward that the absence of sex intergrades in the heterothallic forms is due to the fact that we are here considering gametophytes, whereas in dioecious green plants, in which such intergrades are known, we are considering sporophytes with the diploid complement of chromosomes.

The so-called " imperfect " sexual reactions which are to be found when different species and genera of the Mucorales are contrasted has been reinvestigated by Satina and Blakeslee (1930). The fact that these reactions take place indicates that some common factor is present which elicits the sexual activity between species which may not be closely related taxonomically and which may even belong to different genera and families. This factor would appear to be quantitatively different from the one immediately responsible for the fusion in the perfect sexual reaction.

Contrasts made between homothallic and heterothallic forms also give these imperfect sexual reactions, but it was discovered that different homothallic species behaved in a varying manner in this respect when contrasted with heterothallic species. Some, for example, reacted with both the plus and minus strain of the heterothallic form (*Mucor genevensis*), whilst others (*Sporodinia grandis*) showed no reaction whatever. Others again showed a minus tendency (*Absidia spinosa, Zygorhynchus moelleri* and *Z. vuillemini*) and reacted prominently with the plus form of the contrasting heterothallic species, and one, *Zygorhynchus heterogamus*, showed a plus tendency and reacted with the minus strain of the contrasting heterothallic form. In those homothallic species with a minus tendency it was the small progametangia which reacted with the plus strain of the heterothallic form, whereas in the homothallic species with a plus tendency (*Z. heterogamus*) the small gametangia reacted with the minus strain of the heterothallic species. Hence the generally accepted belief ex the relative size of uniting sexual cells as a criterion of male and in that mu cannot be of universal application.

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and also the lateral hyphæ in the hermaphrodite species were bisexual, but the terminal hyphæ were more active in these imperfect sexual reactions. When a number of contrasts were made between heterogamic and homogamic hermaphrodites and plus and minus heterothallic races, it was found that the former were divisible into two groups. In the first, which included *Absidia spinosa*, *Dicranophora*, *Zygorhynchus moelleri* and *Z. vuillemini* showed a strong minus tendency and reacted vigorously with the plus races, while in the second group, *e.g.*, *Z. heterogamus* showed a plus tendency. Beyond stating a number of facts, it cannot be said that this investigation advances in any way the underlying cause of sex.

Heterothallism has now been shown to exist in other members of the Phycomycetes. In the Oomycetes, Ashby (1922) has demonstrated that *Phytophthora faberi* is heterothallic or dioecious, and Couch, that species of *Dictyuchus* is also heterothallic In both these forms, antheridia and oogonia are borne on separate haploid mycelia.

Ashby's work on the heterothallism of *Phytophthora* has now been extended (1929) to other species than *P. faberi*. It was found that as a general rule the members of this genus did not form sexual organs very readily in single spore cultures. For example, *P. Arecæ* did not develop oospores on any kind of medium, nor did *P. Meadii* (the cause of the leaf-fall disease in rubber), but when these two species were grown together sexual organs were very freely formed in from four to five days. In *P. Richardiæ*, however, sexual organs were formed alone in culture, but their development was more free when it was paired with *P. Cinnamomi*.

Ascomycetes. Heterothallism has now been shown to exist in the Ascomycetes. In 1914 Egerton examined *Glomerella cingulata* in this connection, and found that two strains (designated by him as (+) and (−)) were necessary for the production of normal perithecia. Since this work a number of publications have appeared, showing the necessity of two strains for the production of fructifications in the Ascomycetes.

Ascobolus magnificus was investigated by Dodge (1920), who showed that antheridia and oogonia only made their appearance

along the line of juncture of two different mycelia, and a similar result was obtained by Betts for *A. carbonarius*. Derx has described a heterothallic form of *Penicillium luteum*. *Penicillium* is notorious for the difficulty of obtaining its sexual organs. It was ascertained by Derx that monoascosporous cultures readily and abundantly produced well-developed ascocarps when they were grown two by two in certain combinations. When this point had been fixed, the different strains isolated from conidia behaved in the same way. Shear and Dodge have described a case of heterothallism in the genus *Neurospora*. In *N. tetrasperma* the ascus contains four spores in place of the usual eight, and each ascospore is binucleate. In *N. sitophila*, on the other hand, the usual eight uninucleate spores are produced in each ascus. When *N. tetrasperma* was grown in culture, perithecia were obtained without difficulty, whereas perithecia were only formed in *N. sitophila* at the junction of (+) and (−) mycelia. According to Dodge (1927), and also his co-worker Wilcox, *N. sitophila* presents a true case of heterothallism and the character distinguishing the (+) from the (−) strain being found in the nuclei, and this distinction is brought about in the second division in the ascus.

Gwynne-Vaughan and Williamson have now shown that the discomycete *Humaria granulata* is heterothallic. In this form no antheridia are produced and the archicarp develops as a side branch from an ordinary hypha. The apical cell of the branch swells and becomes spherical and gives rise to the oogonium, which contains a large number of nuclei. In single spore cultures it was discovered that *H. granulata* did not develop ascocarps, but these were formed along the line of juncture of (+) and (−) infections.

The heterothallism exhibited by the Pyrenomycete, *Pleurage anserina*, has been investigated by Dowding. This Ascomycete normally contains four spores in the ascus, and each spore is binucleate and bisexual. Single spore cultures made from these spores fruit readily. At the same time asci are to be found with so-called "giant" spores and "dwarf" spores. The dwarf spores occur in pairs, and each pair replaces a single normal spore, whereas each giant spore replaces two normal spores.

Mycelia grown from single spore cultures of giant spores fruit readily, whereas single spore cultures from dwarf spores remain sterile. It was found, however, that when the dwarf spores were mated in pairs, they were divisible into two groups, designated (+) and (−) respectively. When two plus strains came into contact, no fruit bodies were developed, and a similar result was obtained when two minus cultures came into contact. Perithecia were only developed when a (+) and a (−) race were contrasted. In other words, the dwarf spores are unisexual, and they are also uninucleate.

A curious situation has been described for *Diaporthe perniciosa* by Cayley. This Ascomycete is the cause of " die-back " in fruit trees, and in her preliminary account Cayley described the phenomena of " mutual aversion " between monospore mycelia. She has now carried the matter a good deal further. There are two distinct stages in the life-history : (1) a pycnidial or vegetative spore stage, the pycnidia containing two kinds of spores, oval or " a " spores and filiform or " b " spores ; the " a " spores only being viable ; and (2) a perithecial or sexual stage. Each perithecium contains numerous asci and very evanescent paraphyses, and there are eight bicellular ascospores to each ascus.

In pure cultures perithecia are only sparingly produced, and polysporous cultures of either pycnospores or ascospores produce numerous pycnidia, but few perithecia. Under natural conditions perithecia are also sparingly produced. The heterothallism discovered here was of a complex character, and the author's confused method of presentation of the facts does not make it an easy task to follow every step in the argument.

Cayley found that the capacity for showing aversion between monosporous cultures was inherited and segregated in subsequent generations. In one particular case three pycnospores were sown in the same petri dish, and of the three mycelia produced one showed aversion to the other two. This was sub-cultured and found to produce perithecia. The ascospores from the same perithecium showed aversion *inter se* when cultured for the second and third generation. In other words, *intra*-perithecial aversion was shown. At the same time, from other matings *inter*-racial

aversion was also found between biologic races, and these two types of aversion are macroscopically indistinguishable. Cayley looks upon *inter*-racial aversion as being due to a form of sterility between biologic races, whereas she considers *intra*-perithecial aversion as due to self-sterility, a peculiar form of physiological heterothallism other than sex. She found experimentally that both the homothallic and heterothallic forms of *Diaporthe* were capable of showing *inter*-racial aversion, but *intra*-perithecial aversion was only found in the heterothallic form.

Basidiomycetes. The Basidiomycetes have been the centre of exhaustive investigations on heterothallism during the past decade. In 1918 Bensaude, in France, and Kniep, independently, in Germany, showed quite conclusively that the phenomenon of heterothallism exists among the Hymenomycetes. Bensaude, in making cultures of *Coprinus fimitarius*, obtained four mycelia, each of which was from a single spore culture. Of these four mycelia only two survived when they were transferred to tubes. The two surviving monosporous cultures, which were termed α- and β- by Bensaude, were subcultured, but during eight months they remained in the so-called "primary" condition, *i.e.*, they developed no clamp-connections nor showed paired nuclei and remained sterile. The behaviour of the polysporous cultures, however, was very different. These polysporous cultures were obtained by mixing pieces of the α-mycelium with the β-mycelium, and as a result hyphal fusions occurred and a "secondary" mycelium was formed, in which the nuclei were found to be paired and divided conjugately, the division of each dikaryon being accompanied by wall formation with a clamp-connection, and fruit bodies made their appearance.

The formation of these clamp-connections is somewhat peculiar. The development of clamp-connections takes place simultaneously with the deposition of a cell wall and in connection with the conjugate division of the paired nuclei. When division of the nuclei is about to take place, a projection, rather beak-like in structure, is put out from the cell. This now curves down and fuses again with the cell. In the meantime the upper of the two nuclei approaches the beak, while its fellow remains

in the parent hypha. Simultaneous nuclear division now takes place, and one of the daughter nuclei passes into the beak and associates itself with the daughter nucleus of the other member of the dikaryon. Cell walls are now laid down across the beak as well as the original cell, each at right angles to one of the spindles of the dikaryon. As a result of this somewhat complex method of nuclear and cell division a clamp-connection is formed.

Kniep obtained very similar results to Bensaude for the Hymenomycete, *Schizophyllum commune*, but found that fruiting was not necessarily dependent upon the presence of paired nuclei in the mycelium. For example, a haploid mycelium derived from a single spore culture was able to produce fruit bodies, resembling in external appearance and in the production of several ripe spores the fruit bodies of the same species formed on a diploid mycelium, derived from the fusion of two monosporous mycelia. Thus the mere formation of fruit bodies by a monosporous mycelium is no evidence that the fungus is homothallic. According to Kniep, in a heterothallic species, the difference between the haploid fruit body produced on a monosporous culture and a diploid fruit body produced on a polysporous culture lies in the fact that in each basidium of the haploid fruit body there is only one nucleus when the cell is cut off from the parent sub-hymenial cell, whereas each basidium of a diploid fruit body possesses two nuclei. In the haploid basidium the single nucleus divides twice, while in the diploid basidium there is first nuclear fusion, and this is followed by two divisions.

Mounce considerably extended the observations of Bensaude and Kniep, and discovered the presence of both homothallic and heterothallic strains in the genus *Coprinus*. Thus *C. sterquilinus* and *C. stercorarius* are both homothallic and produce clamp-connections and fruit bodies in monosporous cultures, and *C. lagopus* and *C. niveus* are heterothallic. She was further able to ascertain that the question of sex in heterothallic Coprini is complicated by the fact that the sexual strains cannot be strictly divided into (+) and (−) groups. For example, in *C. niveus* she found that in a series of nine cultures, which may conveniently

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be designated by the letters A to G, A formed clamp-connections with D, D with C, F with E, and B formed clamp-connections with F, H and G, yet when cultures of B and D were grown together no clamp-connections were produced.

According to Kniep, *Schizophyllum commune* and *Aleurodiscus polygonius* possess four sexually different kinds of spores. Kniep assumed that in these strains sex is determined by two allelomorphic pairs of factor which are present in the fusion nucleus of the basidium, and that these became segregated in the two subsequent nuclear divisions according to Mendelian principles. If these factors be represented by the letters (Aa) and (Bb), then the fusion nucleus of the basidium will have the composition (AaBb). At the second division of the fusion nucleus, the sex factors will be separated as (AB), (ab), (Ab) and (aB), and four different kinds of spores will be formed. Only those spores without a common factor will unite sexually in the mycelial stage. Thus (AB) will combine with (ab), whereas (AB) and (aB) will not unite since they both carry the common factor (B). Kniep was able to show that in *Aleurodiscus polygonius* each basidium bears two pairs of spores, one of each sex. He also ascertained that the basidia of a single sexual strain were of two kinds, one kind bearing the spores (AB), (AB), (ab) and (ab), and the other (Ab), (Ab), (aB) and (aB). He concluded from these results that the reduction in chromosome number occurs in the first and not in the second of the fusion nucleus of the basidium.

In an extensive investigation of *Coprinus lagopus*, Hanna (1925) found that the spores from any individual fruit body belonged sexually to four different groups. The basidial analyses showed, however, that though some of the basidia bore spores of two sexes only, a pair of one sex and a pair of another and opposite sex, other basidia bore spores of all four sexes : (AB), (ab), (Ab) and (aB). Since some basidia were obtained with four sexually different kinds of spores on a single basidium of *C. lagopus*, Hanna claimed that the reduction process was brought about in the second, and not in the first division of the fusion nucleus. The views of Kniep and Hanna are shown diagrammatically below :—
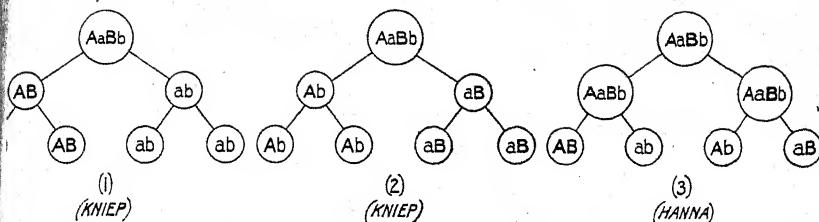
Funke took up a similar attitude to Hanna for *Hypholoma fasciculare*, *H. capnoides* and *Collybia velutipes*, namely, that reduction was deferred to the second division of the fusion nucleus. According to Vandendries (1924), *Coprinus radians* bears two different kinds of spores. All possible crossings were made between twenty-three monosporous mycelia derived from a single fruit body, and, secondly, between twenty-five monosporous mycelia derived from another fruit body. Without exception, in the first experiment, the spores proved to be of two sexes. In the second experiment, twenty-four of the twenty-five proved to be of two sexes, but the twenty-fifth behaved in an anomalous manner and reacted with all the other cultures. In one case Vandendries discovered that twenty-seven of his cultures passed spontaneously from the haploid to the diploid state. From these facts he advanced the view (1925) that all species of *Coprinus* are at first heterothallic. In other words, the spores of the homothallic, as well as the heterothallic species, are at the commencement unisexual, and he has suggested that in the homothallic forms, such as *C. sterquilinus*, the mycelia change at a very early stage in their development from the haploid to the diploid condition, but that in the heterothallic species this development is delayed over a longer period, which may be of several weeks, or even months, in duration. To Vandendries, *C. sterquilinus* is not homothallic, and *C. radians* is not heterothallic, but they show the state to which he has given the name " hetero-homothallic." Brunswik, who has investigated *Coprinus curtus*, *C. deliquescens*, *C. ephemerus* and *C. velaris*, obtained very similar results to those found by Vandendries for *C. radians*, but preferred to explain his observations by the presence or absence of sterility rather than of sex factors.

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Newton has now shown that *Coprinus Rostrupianus* is hetero-thallic. All monosporous cultures during the first few weeks were found to be entirely unisexual, half being of one sex and half of the opposite sex. Of twenty-five monosporous cultures which were kept under conditions of continuous cultivation for six months, eleven remained haploid throughout this period and fourteen passed spontaneously into the diploid condition. *C. Rostrupianus*, unlike *C. lagopus*, is bisexual, and the basidia bear two kinds of spores only, two of one sex and two of the opposite sex. Here sex is apparently concerned with a single pair of Mendelian Factors (Aa). In certain cases the segregation of sex factors was found to occur in the second nuclear division in both *C. Rostrupianus* and *C. lagopus*. The method of proof was ingenious in the extreme. If segregation of the sex factors involved in *C. Rostrupianus*, (A) and (a) take place in the first division, then the two spores bearing (A) should have adjacent positions in every spore tetrad, whereas if segregation takes place in the second division, the two spores bearing (A) should occupy adjacent positions in some basidia and be diagonally placed in others. In an analysis of six basidia, the two spores had adjacent positions in four basidia and diagonal positions in two. In *C. lagopus* there are apparently three types of basidia, a four-sex type: (AB), (ab), (Ab) and (aB); a two-sex type (AB), (AB), (ab) and (ab); and a further sex type (Ab), (Ab), (aB) and (aB). Of these three types of basidia in the hymenium of any fruit body, 50 per cent. are of the first type, and 25 per cent. of each of the other two types. According to Newton, the results discovered here are best explained on two assumptions : (1) that the two sex factors in the nucleus of each spore are carried by different chromosomes, one factor on each ; and (2) that in some basidia the segregation of sex factors takes place in the first division of the fusion nucleus of the basidium, and that in other basidia the segregation of one pair takes place in the first division and of the other pair in the second division.

Some interesting observations have now been made by Sass on certain two-spored forms of the Hymenomycetes. In these species basidia are to be found with the normal complement of

our spores, and others with fruit bodies, the basidia of which only contain two spores. In all, three different species were investigated : *C. ephemerus*, f. *bisporus*, *Galera tenera*, f. *bispora* and *Naucoria semiorbicularis*, f. *bispora*. In the first-named species, clamp-connections made their appearance within two to three days in monosporous cultures and fruit bodies were formed in fourteen days. In 200 cultures of monosporous mycelia, however, 10 per cent. did not develop clamp-connections and 12 per cent. died within a few days. When the 10 per cent. were mated in all possible ways, in only four cases did secondary mycelia make their appearance. Similarly in *Galera tenera* f. *bispora*, except in 4·5 per cent. of cases monosporous mycelia developed clamp-connections, and much the same results were obtained for *Naucoria semiorbicularis* f. *bispora*. Now, the four-spored forms of these three species are definitely heterothallic, and, as far as *C. ephemerus* is concerned, it is a bisexual type and sex is determined by one pair of Mendelian factors. A cytological investigation of *C. ephemerus* f. *bisporus* revealed the fact that the basidium is at first binucleate and that fusion takes place. Nuclear fusion is followed by two nuclear divisions, which presumably constitute a meiotic phase. It was found difficult to trace the ultimate fate of the two nuclei, but the mature spores are multi-nucleate structures, and may contain as many as eight nuclei. There is a good deal of evidence to show that in the two-spore forms each spore may receive two nuclei from the parent basidium. Presumably the two nuclei of the spores would be of different sexes, and each spore would give rise to a homothallic mycelium. On the other hand, Sass obtained definite evidence that in certain cases only one nucleus entered each spore from the basidium. It is possible that this result gives the clue to the behaviour of some of the monosporous cultures which continued to remain in the primary condition, and in order to produce secondary mycelia must be mated with another haploid mycelium.

Heterothallism has now been recorded in the Uredinales. Craigie found that in *Puccinia Helianthi*, when the basidiospores were shed on the leaf of the sunflower, spermogonia made their appearance in about eight days. In ten or eleven days after

sowing, when the mycelia from two different infections overlapped, æcidia were discovered in about 50 per cent. of cases. The rest of the infections, either simple or compound, did not show any signs of æcidia for three weeks. Later, however, approximately 50 per cent. did so. It is clear from these results that the presence of two mycelia, evidently of two different strains, stimulated the production of spore fruits. Craigie was also able to show that when the contents of several spermatia were mixed and added to the infections, in nearly every case æcidia were produced. Similar results were obtained for *Puccinia graminis*, and the two strains involved here are regarded by Craigie as being of two different sexes.

Further investigations on the heterothallism of the Uredinales have been made by Allen. She found that when the teleutospores of *Puccinia graminis* were germinated on barberry leaves, a haploid mycelium was developed and spermogonia were formed four days after infection. It was also ascertained that isolated infections produced as many as two dozen æcidia, but, although these frequently attained a considerable size, they eventually disintegrated and died. It was only when spermatia were transferred to the developing æcidia that they passed into the diploid condition.

Andrus, from his investigations on the sex-mechanism of *Uromycea appendiculatus* and *U. Vignæ*, makes the claim that fertilisation here is similar to that in the Rhodophyceæ. Both these species are full-cycle Rusts, showing little, if any, physiological specialisation. The constant appearance of "two-legged" uninucleate cells was found in the formation of æcidial primordia. In the early stages of the development of the æcidial primordium these cells were found to be set at all angles, but at a later stage those nearest the epidermis of the leaf became disorganised, and in part came to compose the pseudoperidium. These two-legged structures are interpreted as being an oogonium with a foot-cell and trichogyne. With further development, these " trichogynes " become highly septate organs, and their terminations can be seen to project through the stomata or between the epidermal cells. The sex act is said to be brought about by fusion with spermatia,

and actual nuclear migrations were seen passing through the cross-walls of these trichogenous hyphæ and migrating into the fertile cells of the æcidium. Although fusions of parallel hyphæ within the æcidium were observed, these are interpreted as being nutritional or vegetative in nature, or are possibly similar to the fusions of auxiliary cells in the Red Algæ.

The question of whether the stimulating action of the spermatia on the developing æcidia was due to the presence of living material or whether killed material was equally effective was investigated by Craigie, who heated the spermatial exudate to 70° C., and then found that its stimulating properties had been destroyed, and concluded that living material was necessary for stimulating activity. Cummins has advanced the objection that the heat would destroy any enzymes in the exudate itself, and that these enzymes, rather than the spermatia, might have been the possible cause of the stimulating action on the æcidia. He has reinvestigated the matter in an apparently heterothallic Rust, *Puccinia sorghi*, whose alternative host is *Oxalis*. In place of heating the spermatia and exudate, the whole was filtered through a small Berkefeld filter. By this arrangement the spermatia were excluded and only the exudate passed through. Pustules treated with the filtrate failed to form mature æcidia, hence æcidial formation cannot be explained on the basis of enzymic stimulation, since the stimulating activity was lost when the spermatia were excluded, and Craigie was therefore correct in his ultimate conclusion.

Both Kniep (1926) and Dickinson, as well as others, have now shown that the Ustilaginales or Bunt Fungi are heterothallic in certain cases. Kniep, for example, isolated sporidia from a mass of germinating chlamydospores and found that the latter could be arranged in two groups—A and B ; the sporidia in group A would conjugate with those from group B, but no conjugation would take place between members of the same group. Since the numbers in each group were approximately equal, Kniep on this ground considered that segregation of the two forms occurred in the second nuclear division of the promycelium. According to Bausch, *Ustilago longissima* is trisexual, and the sporidia can

be divided into three groups, members of which conjugate with those of either of the others, but no conjugation occurs in the same group.

Dickinson has fully investigated the heterothallism exhibited by *Ustilago levis* and *U. Hordei*. The chlamydospores were allowed to germinate on a 1 per cent. beef extract and the sporidia isolated in turn as they were formed on the promycelium, and each was then transferred to a beef extract medium. It was found that when two mycelia, each derived from a single sporidium, were allowed to meet, one of two things happened: either they grew past each other, or, after approaching to within a certain indefinite distance, they now definitely curved towards one another. At the next stage they came into contact and took on a brown tint, and in the majority of cases the contents of one passed into the other, leaving an empty cell at the point of union. It was discovered by Dickinson that the sporidia of any promycelium could be classed into two groups, termed respectively A and B, and of the four sporidia produced, two belonged to A and two to B.

After fusion had taken place between sporidia, the binucleate cells thus produced, formed hyphæ into which the nuclei passed. After the hyphæ had undergone a certain amount of growth the nuclei underwent conjugate division, with the result that a definite arrangement of the nuclei was brought about: an A passed to one end and a B to the opposite end, and an A and B were associated in the centre.

Nutritive Theory of Heterothallism. In 1928 Dame Helen Gwynne-Vaughan, in her Presidential Address to Section K of the British Association at the Glasgow meeting, advanced an explanation based on nutrition to account for heterothallism. In the Ascomycetes, for example, *Humaria granulata*, which lacks an antheridium, and in which the ascocarp is developed from an oogonium, two strains are necessary before the fruit body makes its appearance. If the (+) mycelium be assumed to be a saltant, becomes, as an hereditary character, possesses the power of extracting to projes sential food substance (A) from the substratum for the The sex act of the ascocarps, but does not possess the power of

absorbing some equally important and essential substance (B), and further, if it be assumed that the (—) strain can extract (B) rapidly, but not (A), then, when the two strains meet, the two essential conditions for ascocarp formation are present, and the fruit bodies are produced in abundance. Similarly in the Hymeno-mycetes, certain forms, such as *Aleurodiscus polygonius* and *Coprinus lagopus,* are described as being quadrisexual. Such a conception is difficult to visualise, and it is equally difficult to imagine a race composed of four sexes, but no such difficulty enters into the conception of a race requiring four different food sub-stances in preparation for the fruiting period. Thus, if the four characters termed (A), (a), (B), (b), which these fungi have been shown to inherit on Mendelian lines, each represent the power of rapidly withdrawing from the substrate some essential food factor, and again, if each spore contain either (A) or (a), and either (B) or (b), then the necessary conditions for sporophore formation will be present when (AB) and (ab) or (Ab) and (aB) meet.

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CHAPTER VI

FUNGI (continued)

MYCORRHIZA

Introduction—Orchidaceæ—Distribution of Fungus in Roots and Rhizomes of Orchids—Nature of Fungus in Orchid Mycorrhiza—Germination in the Absence of Fungus—Physiological Relation between Fungus and Host — Gastrodia — Ericaceæ — Lolium — Bryophyta—Tree Mycorrhiza—Physiological Rôles of Endophyte and Host—Theory of Double Infection.

THE living together of two organisms, *symbiosis*, as it is often termed, is a well-known phenomenon in both the animal and plant kingdom. It is usually thought that the intimate relationship that exists between the partners of such a union results in some mutual benefit to both. The well-known case of the bacteria in the nodules of the Leguminosæ is an example of symbiosis, while the large group of composite plants known as Lichens furnish an even better example. In the latter is a close union between green alga and fungus, with the fungus as the dominant partner of the situation. The marine worm, *Convoluta*, furnishes an example of symbiosis between animal and plant, for in the body of the former a green alga is always to be found. It is not proposed to discuss here the phenomenon of symbiosis in its wider issues, but to consider only the relationship that is known to exist between certain of the higher green plants and the fungi.

The presence of fungal hyphæ in the roots of some of the higher plants has been known for nearly a century, and for a full historical survey of this part of the subject the monograph by Rayner (*Mycorrhiza*) should be consulted. The first observation of the presence of fungi in the roots of higher plants was apparently in 1840, when Link observed the presence of granular masses in the young seedling of *Goodyera prosera*. He was, however, unable

to interpret correctly the nature of his discovery, and considered that they were colourless granular material which finally disappeared. Similarly in 1842 Schleiden observed very much the same thing in the roots of the saprophytic Orchid, *Neottia nidus avis.* He discovered these inclusions to be present in the cortical tissues of the root and made some very excellent and accurate drawings, although, like Link, he was quite unable to interpret his results.

The work of Reissek in 1847 was a real landmark in this matter. He made an anatomical investigation of a number of vascular plants, and found the presence of fungal hyphæ to be present in a number of different species, especially in members of the Orchidaceæ. The forms investigated were both exotic and native genera, and he showed that in the tropical forms the fungus was present in the peripheral region of the roots, whereas in the native form, *Orchis Morio,* it had a considerable extension in the cortex. He also showed that the highest development of the fungus occurred in underground roots, the least in the aerial. More remarkable still, Reissek attempted to isolate the fungus from orchid roots. From *O. Morio* he obtained a *Fusisporium.* This was probably a *Fusarium,* saprophytic in the soil; and he also obtained from other orchid species a *Botrytis, Penicillium* and *Cladosporium.* When the fact is considered that mycological technique, such as we know it now, was at that time non-existent, it is not surprising that he should have failed in his quest; it is the more remarkable that he should have made the essay at all.

In this country, T. G. Rylands in 1844 described the presence of certain "byssoid" substances in the roots of *Monotropa Hypopitys.* He concluded that this byssoid substance performed no particular or essential function in *Monotropa.* It is therefore to the Continental workers that we owe our first real advances in this subject.

Another kind of association of fungus and root was also observed as far back as 1840, when Hartig noted that the extremities of the roots of *Juniperus* and *Thuja* and also *Pinus sylvestris* were clothed with a mantle of fungal mycelium. Unfortunately, he mistook their real nature and considered that the hyphæ were intercellular

canals surrounding the corky layers of the root cortex. Similarly, sixteen years later, in 1856, Gasparini observed that the rootlets of *Corylus* and *Castanea* were surrounded by fungal hyphæ.

Our present knowledge of the subject may be said to commence with the investigations in 1885 of Frank on the roots of *Neottia nidus avis*. Frank coined the name *mycorrhiza* for this association of fungus with roots. He also distinguished between the two types mentioned above and termed those forms in which the fungus was lodged within the root *endotrophic* mycorrhiza, and those forms in which the fungus formed a covering on the extremities of the root exterior, *ectotrophic* mycorrhiza. The term is now employed in a very much wider sense than that originally meant by Frank. Thus, for example, the presence of fungus within the thalli of Liverworts is termed mycorrhiza. This, it is true, is etymologically unsound, but has the merit of convenience.

Mycorrhiza is now known in Angiosperms, Gymnosperms, Pteridophytes and Bryophytes. Such an association is of very great antiquity, and the fossil forms from the Chert of Rhynie (*Rhynia*, *Hornea* and *Asteroxylon*), which geologically belongs to the Middle or Lower Devonian, have their attendant fungus. It is equally possible that these are not mycorrhiza at all, but saprophytes that lived on the dead remains of these plants. Nevertheless, the observations of Weiss on roots from the Lower Coal Measures have shown quite clearly that such a union existed even at such an early date as the Lower Carboniferous. It will be necessary to consider the better-known advances that have been made on mycorrhiza in different groups and families of plants. The first of these that calls for consideration is the Orchidaceæ.

Orchidaceæ

The orchids as a class produce an enormous number of seeds from each capsule. Charles Darwin estimated that in a single capsule of *Cephalanthera grandiflora* 6,020 seeds were present. Many of the tropical forms contain even greater numbers; Scott estimated the number as 371,250 in *Acropera*, and Muller arrived at the huge total of 1,756,440 for *Maxillaria*.

The seed of an orchid is a minute dust-like structure. It possesses a single integument in the form of a very characteristic network which shows a certain amount of variation in different genera and species. The embryo is not differentiated into cotyledon, plumule and radicle. As a general rule the cells at the suspensor end of the seed are larger than at the upper end, although this is not always the case. In *Cattleya* the suspensor cells are permanent, while in *Phalænopsis* they disappear before the maturation of the seed.

The great difficulty of germinating orchid seeds has been known for a number of years. Horticulturalists discovered the fact that germination was more successful if the seed were sown in soil in which the parent plant had been living, but this was only an empirical custom and the reason was quite unknown. It remained for the Frenchman, Noel Bernard, to produce convincing evidence that unless a suitable fungus was present little or no germination would take place.

The investigations of Noel Bernard began in 1899, and finished at his death in 1911. The first orchid investigated by him was the saprophyte *Neottia*, and in 1902 he announced that an orchid seed can only germinate in the presence of the root fungus, and that the seedling is infected from the earliest stages.

Orchid seeds, when removed from the capsule and sown on ordinary substrata in which no fungus is present, do not, as a rule, germinate. In *Odontoglossum* the seeds merely swell and develop chlorophyll and become green. *Cattleya* develops rudimentary hairs and forms a few stomata, while the seeds of *Epidendrum* do not even swell and become green. According to Bernard, the greatest development takes place in *Bletilla hyacinthina*, in which weakly seedlings are formed with definite leaves. If no fungus infection takes place the seedling dies

Bernard germinated seeds under aseptic conditions. The capsules were sterilised with formalin, and the seeds were then removed under aseptic conditions to tubes containing a gelatin decoction of salep. A control was set up in the greenhouse, using ordinary horticultural conditions. It was found that seeds sown

in the greenhouse germinated, while those in the tubes failed to do so.

Germination in the presence of the fungus takes the following course. Entry of the fungus occurs at the suspensor end of the seed. The invasion of these cells takes place by degrees, and the hyphæ become twisted into a ball in each cell before passing on to the next. The presence of the fungus in some way stimulates the smaller cells at the end of the seed opposite the suspensor, and these proceed to divide. It is within this region that the stem meristem is laid down, and it should be noted here that the fungus never penetrates the meristematic cells. Finally, the seed takes on a swollen shape.

Distribution of Fungus in Roots and Rhizomes. It has already been seen that the presence of fungal hyphæ in the roots and rhizomes of orchids has been known for a very considerable time. Investigations have now shown that they are present in every case with the exception of the saprophyte *Wullschlægelia aphylla.*

The entrance of the fungus in the root has been carefully followed by different investigators. As a general rule it is absent in the epidermal layer. Entrance is effected through a root hair, or even occasionally through the piliferous layer itself. The hyphæ pass through to a more or less definite zone, in which they attain their highest development and rapidly fill the cells. Should an exodermis be present, the fungus passes through the passage cells. The position of the fungal zone varies from genera to genera. In *Habenaria,* for example, the hyphæ usually occupy the third and fourth layer of cortical cells, whereas in *Neottia* and *Epipogum* the hyphæ occupy three layers or so of the cells separated from the endodermis by half a dozen layers of cells. *Cymbidium* and *Odontoglossum* show an extreme development of the endophyte which is distributed throughout the cortical area. It should be noticed that the fungus never passes the barrier of the endodermis and the vascular region is always uninfected.

The changes that take place in the fungal hyphæ in their invasion of the root cells have been closely followed by Wahrlich, Magnus, Bernard and Burgeff. In *Neottia* it was shown by

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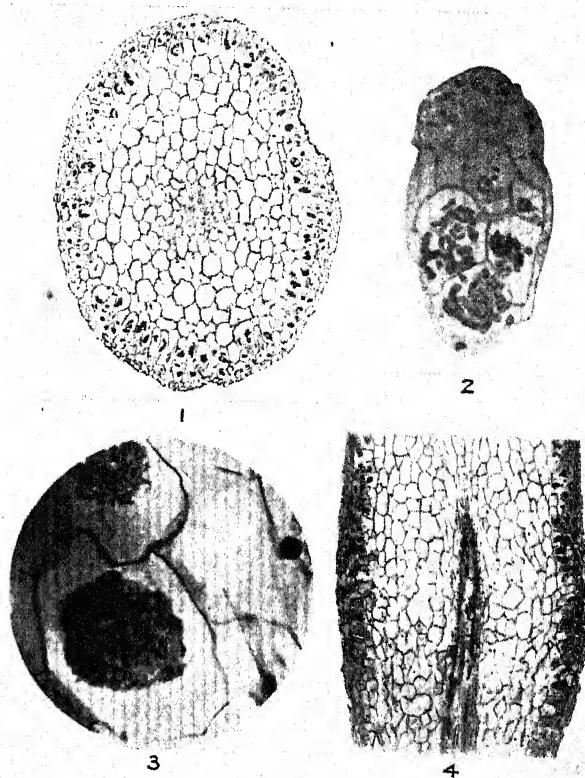
Fig. 43.—(1). Transverse section of root of *Habenaria* just above the root tip. The dark masses show where digestion of the fungus is taking place. (2). Longitudinal section of a seed of *Odontoglossum* nine days after sowing. The fungus has entered the larger cells at the suspensor end of the seed and formed balls of hyphæ. (3). Cells from fungal zone of the first root of *Odontoglossum*. The fungus is "clumped" (*cf.* 2). A fungal hypha is seen passing through the cell wall. (4). Longitudinal section of an aerial root of *Epidendrum* showing infected cells in the centre and mycelium in the velamen. (After Ramsbottom, *Charlesworth & Co.'s Catalogue*.)

Magnus that two main types of infected cells are present, and he considered that there were no transitional stages. In the first type, which he termed " digesting cells " (Verdauungszellen), the fungus always degenerates, while in the second type, which he

called " host cells " (Pilzwirthszellen), the fungus remained alive in the cells which lodged it, and could under these conditions hibernate. According to Magnus, in *Neottia* these two types of cells show a definite arrangement. The outer region of the root cortex is composed of digesting cells and the inner zone of host cells. It is doubtful, however, if such a regularity of arrangement of these two different types of cells is really present.

Bernard and Burgeff have also studied this problem of fungus entry. In general terms it can be stated that when a hypha enters a host cell the nucleus of the latter increases in size. It undergoes considerable changes in shape and sometimes becomes hypertrophied, and may even in certain cases disintegrate, thereby showing that the fungus can sometimes play the part of a parasite. The digesting cells can be very readily seen by the degenerating mass which fills them. The nuclei of these cells increase very much in volume and often become amœboid and put out pseudopodia which attack the entering hyphæ. The pseudopodia which are put out by the nucleus of the digesting cells now enclose the entering hyphæ, which soon lose their characteristic outline and become converted into amorphous, yellow masses, which are totally devoid of life.

The Nature of the Fungus in Orchid Mycorrhiza. In his first attempts to isolate the fungus from orchid roots, Bernard obtained a *Fusarium*. Later, however, he obtained the true endophyte, and was able to grow it in culture. It was found that the fungus " balled " up on the artificial medium in much the same way as in the cells of the orchid root. From the fact that a number of swollen segments made their appearance, and were apparently heavily charged with food reserves, Bernard at first placed the endophyte in the genus *Oospora*. The filaments, however, also ramified and formed dark sclerotia, much as in *Rhizoctonia violacea*, and on this account he later came to the conclusion that the endophyte belonged to this genus. It would serve no useful purpose here to give a detailed discussion of the taxonomics of the endophyte, and for convenience it will be termed *Rhizoctonia*. Burgeff, like Bernard, was also successful in isolating the endophyte, and proposed the new class, *Orcheomyces*, for its

reception. Bernard later recognised Burgeff's species as falling within his own, and were regarded by him as *Rhizoctonia repens.*

Bernard found that the efficacy of the endophyte to bring about germination was impaired by prolonged cultivation without the host. Burgeff, on the other hand, was quite unable to substantiate this statement. It is possible that Bernard's cultures were not renewed sufficiently often, with the result that "staling" had occurred.

Germination in the Absence of Fungus. Bernard tried the effect of solutions of sucrose and salep in different concentrations on the seeds of *Bletilla*, *Lælia* and *Cattleya* in the absence of the endophyte. In certain concentrations there was germination, and in higher concentrations seedlings were obtained which could be transplanted.

This aspect of the subject has been considerably extended by the investigations of Knudson (1922, 1924, 1925). In his first paper, seeds of a hybrid orchid (*Cattleya Schrœderæ* × *C. gigas*) were carefully sterilised in weak calcium hypochlorite solution, and sown on either Pfeffer's medium or a modification of this in 1·7 per cent. agar. The addition of fructose to the medium gave the best germination, while the presence of glucose tended to give rise to chlorosis. Plant extracts were also found to answer the purpose. This work was later extended to other species. A *Cymbidium* hybrid was sown on modified Pfeffer's medium with the addition of 2 per cent. of sucrose. Germination was found to be rapid. Similarly, *Odontoglossum Rossii* × *Odontioda* gave positive results. *Phalænopsis Schilleriana* on a modified Pfeffer's medium, and in the presence of either 1 per cent. glucose or sucrose, gave positive results. *Ophrys* and *Dendrobium* also germinated in the presence of glucose and sucrose.

Knudson further found that the fungus was capable of bringing about germination on a medium containing starch and accelerated germination in Pfeffer's medium and sucrose. One of the most important factors controlling germination was the concentration of the starch in the external medium. The fungus completely hydrolysed the starch to soluble sugars and the pH of the medium became altered from an unfavourable to a favourable value.

Moreover, it was ascertained that germination took place in the presence of the fungus, although the seeds were not penetrated. This was found to be due to the change in the *p*H of the medium. Lastly, Knudson was able to bring about germination in a mixed medium of peat and sphagnum plus Pfeffer's modified medium, provided that the *p*H was adjusted to 4·6 and the germination was as rapid as in the presence of the endophyte. Knudson also discovered that *Phytophthora* (sp.) as well as other fungi were as suitable as *Rhizoctonia* for the germination of orchid seeds. The theoretical implications of this work will be discussed below.

The Physiological Relation between Fungus and Host. Bernard considered that relation between orchid and endophyte was similar to that of host and parasite. In other words, the orchid suffers from a benign cryptogamic disease, and the symbiotic relationship between the two represented for him the immunity realised by phagocytosis. There is much to be said for this view. He laid great stress on the presence of tubercles on the seedlings, and thought that these were a direct consequence of fungal infection. Burgeff, on the other hand, held that the function of the fungus was to hydrolyse polysaccharides to simpler sugars which aided germination, while Knudson has taken a very extreme view of the case. From his experimental observations, Knudson arrived at the conclusion that the necessity of fungus infection has not been proved, and that the germination of orchid seeds is dependent on an external supply of organic material, such as soluble carbohydrates, and, lastly, that orchid embryos, when allowed to germinate under aseptic conditions in the absence of sugar, are unable to assimilate photosynthetically because they lack some internal factor. Knudson objected to the " so-called symbiotic theory of germination," and considered, from the fact that the endophyte extracted by himself from *Odontoglossum* rapidly killed *Odontoglossum*, that these fungi are extremely pathenogenic. To Knudson the supreme fact of importance in orchid germination is the supply of soluble and suitable sugar, for the orchid seed is lacking in food reserves, and some external source is necessary to carry the embryo over the critical period until it is in a position to synthesise carbohydrates for itself.

It is unfortunate that Knudson should have taken up the position that the fungus is quite unnecessary for germination, and that symbiosis between higher plant and endophyte as usually recognised, does not exist. His work is really an extension of that of Bernard's, who also found that germination would take place in the absence of endophyte and in the presence of sugars, and that in many cases this method gave more certain results than the use of the fungus. Although it is undoubtedly true that orchids can be grown for many years under conditions in which infection by the endophyte has been completely excluded, this does not explain the fact that under natural conditions the endophyte is always to be found in the roots of these plants. Constantin and Magrou have criticised Knudson from this standpoint, and have pointed out that his work merely confirms Bernard's discoveries, and is no explanation of the presence of the endophyte in naturally growing orchid roots.

Ramsbottom (1929) has also called in question Knudson's views on the symbiotic relation between host and endophyte in the Orchidaceæ, and suggests that the latter's interpretation of non-symbiotic germination of orchid seeds as being due to stimulation by the production of sugars by many fungi is not supported in orchids growing under natural conditions. The fact that the endophyte always penetrates the orchid seed at the suspensor end, and that the cells at the opposite end do not become infected, and, further, that seeds which adhere to the sides and tops of the culture flasks only germinate when the hyphæ reach them, coupled with the fact that he has never observed a young rootlet to become infected until it has entered the soil, supports his belief in the symbiotic theory of germination. To the present author it would appear that a good deal more work must be accomplished before any very definite pronunciation can be given on these conflicting opinions. When a theory has been held for a long time it becomes a difficult matter to abandon it, even in the face of new evidence.

It has usually been denied that the orchid endophyte can fix atmospheric nitrogen. Burgeff, for example, was quite unable to obtain any evidence for this. Wolff, however, has recently

brought forward evidence to show that the endophyte of *Neottia* is capable of fixing atmospheric nitrogen in pure culture.

Gastrodia

Some years ago Kusano described a peculiar and interesting case of mycorrhiza for the saprophytic orchid *Gastrodia elata*, which has a wide distribution in Japan and occurs in woods under *Quercus serrata* and *Q. glandulifera*. The vegetative portion of the plant consists of a tuberous rhizome some 10 to 17 cm. in length. This tuber is oblong in shape and composed mainly of parenchymatous cells. Vegetative multiplication takes place by means of off-sets, and at the end of the autumn the mother body and pedicel of the off-set undergo degeneration, with the result that the young tubercles are set free. Kusano showed that unless the off-sets were infected with the necessary fungus they decreased in size with successive generations, and eventually were unable to multiply any further. The fungus concerned here was the root parasite *Armillaria mellea*, the so-called "honey fungus." *Armillaria* is a particularly de-structive parasite of trees, and possesses black rhizomorphs composed of compacted mycelium. It was shown by Kusano that the *Gastrodia* tuber is attacked by the rhizomorph and infection is effected by a sucker-like branch of the strand which enters the cortical layers. The rhizomorph creeps over the surface of the tuber and suckers enter the outer cells at intervals. The infected area of the tuber is approximately divided into three regions of cells. In the first there is a densely tangled mass of comparatively thick-walled hyphæ, in the second or middle region, the hyphæ are generally thin-walled and often arranged as a pseudoparenchyma, whilst in the innermost region, which is composed of large cells, a few slender hyphæ are to be found. Characteristic alterations occur in the hyphæ in each of these regions. In the first they are permanent, in the second they undergo disorganisation, and in the third they are consumed by the host cells. Unless a tuber be infected by the fungus, flower heads are not formed. Occasionally it was discovered that the

fungus could play the part of a parasite and the tuber was consumed.

At present there is no information available about the germination of the seeds or whether a *Rhizoctonia* be concerned in this process.

More recently McLuckie has investigated the physiology of *Gastrodia sesamoides*, an Australian species. Like *G. elata*, this Australian orchid also possesses a tuberous rhizome with a few scale leaves. The flowering axis, about 18 inches high, is developed from the apex of the rhizome.

Bacterial infection of the tubers was found to be profuse, and the bacteria were able to assimilate free nitrogen. Fungal infection, on the other hand, was confined to a few superficial cells. The hyphæ of the endophyte, which extended from the superficial cells to the soil, were found to be nonseptate, and, although intracellular in distribution, were not consumed by the host cells.

It has been usual to class *G. sesamoides* as a root parasite, but McLuckie disagreed with this suggestion, and considered it to be a case of obligate symbiosis in which the plant is supplied with nitrogen by the bacteria and carbonaceous food by the fungus, and the latter also functions as the water and mineral absorber from the soil. Certainly *G. sesamoides* forms a marked contrast to *G. elata*.

Ericaceæ

It was first suggested by Frank that the Ericaceæ, like the Orchidaceæ, might show mycorrhizal characteristics. In certain members of the Ericaceæ he commented on the absence of root hairs, the reduction of the root-cap and the absence or great reduction in the cortical tissues of the root, and finally on the presence of fungus mycelium in the enlarged cells of the piliferous layer.

The first adequate investigations on this group were by Ternetz, who was able to isolate the endophyte, and showed that it was a member of the Fungi Imperfecti. She also demonstrated that infection of the seedling of *Calluna vulgaris*, the common ling,

occurred through the seedling, and considered that it was able
to fix free nitrogen.

Rayner (1915, 1916, 1922) has conducted an extensive number
of observations on the mycorrhiza of the Ericaceæ. In *Calluna
vulgaris* she showed that germination of the seeds was entirely
dependent on the presence of the endophyte, and that there
was an obligate symbiosis present of a very similar type to that
of the Orchidaceæ. Unlike the Orchidaceæ, however, the endo-
phyte is said to be present through the whole plant, and infection
of the seeds takes place in the ovary. Delicate branched hyphæ
are present in the cells of the ovary wall, in the tissue of the
central column, and also the funicles of the seeds.

Seeds sterilised in weak mercuric chloride solution were found
to give only feeble germination. A few chlorotic leaves were
developed, but no root system, and the seedling eventually perished.
If at this stage it were supplied with the endophyte, normal
development took place. Infection of the seedling root took place
immediately after it emerged from the testa, and the entering
hyphæ forced their way in between the cells of the root apex.
The mycelium now became intracellular and rapidly spread from
cell to cell, being distributed eventually throughout the length
of the plant, and was to be found in stem, root, leaves and
reproductive organs.

Rayner has recorded the presence of ovarial infection in other
members of the Ericaceæ, *e.g.*, Rhododendroideæ, Arbutoideæ,
Vaccinoideæ and Ericoideæ. Presumably, here also there is a
wide distribution of the endophyte throughout the tissues of the
plant.

According to Rayner (1929a), *Vaccinium* presents a unique case
among the Ericaceæ, and exhibits the most highly evolved symbi-
otic relationship discovered in this family. Stahl has specifically
stated that the roots of *Vaccinium myrtillus* are free from fungus
infection, and that plants can be raised from untreated seeds
in sterilised soil. Rayner found it impossible to obtain seeds
free from fungus infection by sterilisation and removal of the
testas prior to germination. All the tissues of the emerging
seedling were subject to invasion by the fungal mycelium, and it

was probable that the normal germination of the seeds was bound up with this invasion. As in *Calluna*, the fungus was distributed throughout the plant, and was to be found in roots, stem, leaves, and extended to the ovaries and other organs of the flower.

Arbutus unedo, the Strawberry Tree, has been investigated by Rivett. Rivett described two different and distinct types of root for this plant, long roots which form the main root system, and root tubercles which are arrested laterals and sub-laterals. In the long roots there is a superficial network of mycelium, and a few of the hyphæ show penetration, but the intracellular extension of the endophyte is small. In the tubercles, on the other hand, there is profuse development of the endophyte, and its presence apparently is the cause of the stunting exhibited by these organs, which are imperfect developments of young root laterals. According to Rivett, the marked differences which are found in the growth of the endophyte associated with the long roots and tubercles respectively, is influenced by the presence of considerable quantities of mucilage on the emerging laterals, and also by excretions from the ruptured tissues of the parent root. A somewhat peculiar characteristic of the tuberous mycorrhiza in *Arbutus* is the formation of stiff bristle-like setæ by some of the hyphæ in the outer region of the fungal sheath. In appearance they are very similar to root hairs, and are only to be found on the tubercles. Rayner claims that ovarial infection occurs in this genus.

Mycorrhiza has been recorded for other families in the Ericales. In the Pyrolaceæ, Henderson has pointed out that a definite series can be traced, showing a gradual passage from species with relatively poor production of mycorrhiza to others with heavier infection, and that there is apparently a relationship between saprophytism and increase in mycorrhiza. Thus in the genus *Chimaphila*, *C. umbellata* shows the feeblest infection, whilst the greatest infection is to be found in *C. maculata*. In *Pyrola rotundifolia* and *P. elliptica* all the cells of the piliferous layer of the root are infected, and there is the beginning of intertwined hyphæ round the root tip. In *Monotropa Hypopitys* there is an

increase in the width and extension of the hyphal sheath and a demarcation into two regions, and in *M. uniflora* there is still greater extension of the fungal sheath.

Christoph has controverted the findings of Rayner with regard to the necessity of the root-fungus for germination in the Ericales. The matter was tested in two ways. In the first series of experiments, cuttings of *Calluna vulgaris* were taken, and these were planted in shallow pots of humus heath soil—the soil in one being sterilised and the other not. In both experiments a number of cuttings struck, and it was only cuttings from unsterilised soil that became slightly infected, whereas no endophyte was present in the roots of the cuttings growing on sterilised soil. Experiments with *Erica carnea* gave similar results. Germination experiments with sterilised and unsterilised seeds gave good results in both cases, and he failed to find capsule infection.

In the Pyrolaceæ he ascertained that in *Pyrola uniflora*, *P. secunda*, *P. minor* and *P. rotundifolia* the infecting fungus possessed clamp-connections, and therefore was probably a Basidiomycete. Using the seeds of *P. rotundifolia*, he discovered that the presence of a fungus was unnecessary for germination, and that the best conditions were obtained when strong concentrated soil extract was used, together with the addition of peptone solution, sowing on humus from habitat of the plants, and keeping the cultures in the dark with moderate moisture. It was thought that organic compounds of the highly concentrated soil solution, acting in conjunction with the peptone, brought about germination by some kind of chemical action. In *Monotropa* the fungus did not possess clamp-connections, and is presumably different to that in the genus *Pyrola*.

Christoph considered that the Ericales presented a case of facultative and not obligate symbionts, since species growing under natural conditions always possess the endophyte in their roots. In very dry places, however, the endophyte may disappear from the roots of *Calluna vulgaris*.

Rayner has submitted this work to detailed criticism. The burden of her argument, however, is that Christoph failed to sterilise his seeds thoroughly. Nevertheless, if Christoph's results

—that cuttings struck on sterilised soil never showed the presence of the endophyte in the roots—be correct, Rayner's view that the fungus is widespread throughout the aerial tissues of the plant falls to the ground and ovarial infection becomes impossible.

Further evidence in support of Christoph's views has recently been presented by Knudson (1929). The latter has suggested that the abnormal appearances shown by Rayner's seedlings in the absence of the endophyte were due to the fact that the nutrient solution in which the seedlings were grown was toxic to them, or, alternatively, that the use of mercuric chloride as a sterilising agent led to injury of the embryo. He repeated Rayner's work, using the same conditions as those he employed for the germination of orchid seeds (see above). Rayner's nutrient mixture,[1] with the addition of 1·5 per cent. of agar, was used for the growth of the seedlings. The pH was adjusted by the addition of either hydrochloric acid or sodium hydroxide. In certain cases 2 per cent. of glucose was also added to the cultures.

In the first series of experiments no attempt was made to separate the seeds from the floral tissues, and calcium hypochlorite was employed instead of mercuric chloride as a sterilising agent. It was found that all the tubes showed contamination, but the fungus proved to be an *Alternaria* and not *Phoma*. The growth of the seedlings was very erratic, the best seedlings selected for examination showing no infection of the roots, and in all cases the roots were perfectly healthy.

A second series of cultures were now initiated, and the seeds were carefully separated from any adhering tissues and sterilised in calcium hypochlorite. The pH of the culture medium varied from 4·5 to 6·6. It was found that no one hydrogen-ion concentration gave any marked appearance of optimum growth. No hyphal growth was found from the surrounding testa, and the agar was quite free from all fungal contamination.

Knudson has put forward the suggestion that Rayner's results in the absence of the endophyte may well be due to the presence

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[1] Rayner's culture medium was made up as follows : KNO_3, 1·0 gm. ; $MgSO_4$, 0·4 gm. ; $CaSO_4$, 0·5 gm. ; $CaH_4P_2O_8$, 0·5 gm. ; NaCl 0·5 gm. ; $FeCl_3$, trace ; H_2O, 2,000 c.c.

of excess of iron in her nutrient medium, and that possibly mercuric chloride was too drastic a sterilising reagent.

Rayner (1929b) has replied to this report, and claims that calcium hypochlorite is not effective as a sterilising agent for *Calluna* seeds, owing to their buoyancy and nature of the testa. Nevertheless, this is not a convincing argument, nor is her second contention, that, owing to the fineness of the mycelium in its early stages of growth, it is very difficult to observe and may well have been missed by Knudson. The latter specifically states that he examined many hundreds of sections, and it is almost inconceivable that if any fungal hyphæ had been present they should all have been missed or washed away on the slides.

Lolium

The grass *Lolium* presents an interesting case of mycorrhiza, somewhat similar in general features to that described for *Calluna* by Rayner. It has been known for a number of years that *Lolium temulentum* contains a layer of fungal hyphæ situated between the aleurone layer and the fruit and seed coat. McLennan examined a number of seeds of *L. temulentum* and *L. perrene*, and found that in every case fungal infection was present. The fungus is clearly endophytic and occurs within the cells. It is present in the embryo-sac either at, or immediately after fertilisation, and it increases in quantity at the expense of the nucellus and the cells of the carpel wall. The fungus is absorbed when the endosperm is formed as a source of food supply for the developing embryo.

At germination the fungus follows in the wake of the development of the stem apex, and is mainly to be found in this region, although it extends for a small distance down into the stem. When the inflorescence is formed the hyphæ are especially abundant at the base of the carpels. It was at first considered that the fungus involved here was a member of the Smuts (Ustilaginales), but in her more recent publication McLennan has shown that it is a Phycomycete.

In a more extensive investigation McLennan (1926) has followed the development of the endophyte in greater detail and, contrary to the former view that it was confined only to the aerial portions

of the grass, has found it to be present in every part of the plant. It is not so well marked in the older roots as in the finer ones. Entry of the endophyte is either through a root hair or directly through the epidermis. When it reaches the interior of the root, the hyphal filament twists in spiral fashion in the lumen of the epidermal cell. The direction of growth in this first region is at right angles to the root surface, and before infection in the next layer there is considerable extension of the hyphæ in a horizontal direction in the root. The nuclei of the infected cells increase in size, and this increase in volume is maintained after the host cells have killed the entering fungus. With further entry, sporangioles and arbuscles are formed. These constitute the most important organs of the endophyte. The arbuscles are of very short duration, and then develop into sporangioles. They contain a large amount of oil in their interior, and in general appearance they show a papillated surface. When conversion of an arbuscule to a sporangiole has taken place, the oil within no longer blackens with osmic acid. Later in the life of the grass, when the sporangioles have reached maturity, they disrupt, owing to some unknown cause, and their content of oil is freed into the cell of the host. Here, again, the oil does not blacken with osmic acid.

The fact that oil is first collected and then discharged into host cell would seem to indicate that the relation between endophyte and green plant is one which leads to the exchange of fat or oil. The idea that there is a mutual advantage between green plant and fungus is not borne out in *Lolium*; rather does it seem that the green plant is the aggressor, and obtains part of its supply of carbonaceous food at the expense of the fungus.

Bryophyta

It has been known for a great many years that thalli of the Bryophyta in a number of cases contain fungal hyphæ. As far back as 1889 Cooke recorded the presence of a fungus (*Cladosporium epibryum*) in certain mosses. But even earlier than this (1879) Kny had recorded the fact that fungal hyphæ were present

in the rhizoids of *Marchantia* and *Lunularia.* More recently Ridler has made a thorough investigation of *Pellia epiphylla.* It was found that the endophyte (a *Phoma*) only occurs in the lower portions of the thallus in the central thickened region. When new growths are formed by the branching of the thallus the fungus is only found in the rhizoids and epidermal tissues.

The hyphæ apparently enter through the epidermal cells, in which they branch very freely, and gradually extend to within 2 mm. of the growing point. In some of the cells the hyphæ show considerable swelling at their tips, and also intercalary protuberances make their appearance. Large spherical and oval-shaped bodies also appear on the hyphæ. These are possibly of the nature of chlamydospores. In several cases the fungus was found to be present in close proximity to the antheridia. It was only after considerable search that the fungus was found in the sporophytic generation, and here it was distributed in foot, seta and capsule.

The effect of the fungus on the gametophyte is very well marked. When first infected, the cells of the thallus possess a number of chloroplasts and show well-marked protoplasmic contents. As infection progresses, these cells become brown and discoloured and the chloroplasts disappear. The fungus appears to be the dominant partner and obtains its metabolic supplies at the expense of the host, damaging it to a certain extent by killing cells which it enters, but the liverwort still retains the power of growth and reproduction, and is able to control the endophyte to some degree, for starch in the cells is replaced by oil. Apparently the entry of the fungus is brought about by purely mechanical means.

In the sporophyte the action of the fungus is more drastic, and it has a twofold effect upon the tissues. The cells turn brown and the chloroplasts are destroyed, and ultimately the cells die. The second effect is that the cell walls undergo partial disintegration, especially the walls of the capsule. Moreover, the endophyte is not confined to any particular region of the sporogonium, but indiscriminately infests all the tissues. Ridler considered that definite symbiosis exists between fungus and the

gametophytic generation of *Pellia*, but in the sporophyte the situation is probably one of parasitism.

Magrou has also investigated the mycorrhiza of *Pellia*. He found that the strongly growing fertile fronds were immune from infection up to the time of spore dispersal and became susceptible to the entrance of the fungus at a later time. The oldest fronds showed profuse infection of the endophyte and, like *Lolium*, there was a conversion of arbuscules to sporangioles. Here the activity of the fungus was in some way controlled by the host. In the younger fronds there was a protective zone at the base of the developing sporogonium, which brought ·about immunity to infection by destroying the main hyphal branches. Magrou obtained his experimental plants from a highly acid soil (pH 4·85), whereas Ridler found in his case that the soil had a pH of 6·8 to 7·0, *i.e.*, it was nearly neutral. A possible explanation of this discrepancy may lie in the fact that the endophyte can control the hydrogen-ion concentration of the cell sap of the gametophyte.

Some interesting observations have been made by Chaudhuri and Rajaram on an Indian species of *Marchantia* (*M. nepalensis*) which grows in the neighbourhood of Lahore. The thallus invariably showed fungus infection. The mycelium was only present in the gametophyte, and was confined to a zone of cells below the air-chambers. The endophyte was isolated and grown on various artificial media, and it was discovered that if it were deprived of asparagin little effect was produced, whereas if maltose were removed, growth of the mycelium quickly came to an end. Although the thallus is able to grow in the absence of the fungus, the sporophytes that were produced quickly died off. It is supposed that this is a case of reciprocal symbiosis. The green plant supplies the fungus with carbohydrates and the specific endophyte is necessary for the formation and maturation of the sporophytic generation.

Tree Mycorrhiza

A number of important investigations by Melin on the mycorrhiza of trees falls to be recorded here. It was originally shown by Muller that if seedlings of the mountain pine were used

as " nurse " plants in the spruce woods of Jutland, they produced a remarkable influence on the latter. Prior to this procedure it had been found that the spruce trees rarely reached maturity. The young plants flourished for a time and then showed lack of growth and died back at the tops. Muller came to the conclusion that the mycorrhiza of the pine was the solution of the problem, and was the chief factor which brought about the improvement of the spruce. He examined the roots of the spruce and found that there was only one kind of mycorrhiza present ; this was an ectotrophic form with racemose branching, whereas in the pine an endotrophic mycorrhiza with dichotomous branching was present in addition to the ectotrophic form. He argued that the endophyte of the pine was able to fix molecular nitrogen and supplied organic nitrogen to the spruce. Unfortunately, he was unable to prove this fact.

Melin, in a long series of papers, which can only be briefly dealt with here, has added materially to our knowledge of tree mycorrhizas. In *Pinus sylvestris* and *Picea abies* he discovered that the differentiation of the root system into long and short roots was dependent on fungus infection, because only the actively absorbing roots became mycorrhizas. In *P. sylvestris* three different types of mycorrhiza were distinguished : (a) *Gabelmykorrhiza*, (b) *einfach Mykorrhiza*, and (c) *Knollenmykorrhiza*. The first type is the most common, and is developed in woodland soil with large supplies of humus. Its presence leads to the arrest of growth of the roots and the production of abundant dichotomous branching. The colour is most usually a golden brown. Knollenmykorrhiza leads to the production of small tubers and presents a greyish colour. The fungi associated with this type of mycorrhiza are *Boletus luteus*, *B. granulatus*, *B. variegatus* and *B. badius*.

In *Picea abies* two types of mycorrhiza have been described by Melin, racemose and simple. The former is usually present under favourable soil conditions, and both types are susceptible to a good deal of morphological alteration owing to the attack of a parasitic fungus, named by him *Mycelium radicis atrovirens*.

In the Knollenmykorrhiza of the pine the individual roots of the tubers are characterised by the presence of large cells in the

pseudoparenchymatous mantle surrounding the roots. There is also a considerable development of endotrophic infection in the cortical region and active digestion of the intracellular mycelium. Knollenmykorrhiza has not been found in spruce (*P. abies*). Knollenmykorrhiza yield fungi which all belong to the same genus. They showed paired branching of the mycelium in the young condition, and clamp-connections were also present. The whole group were given the name *Mycelium radicis sylvestris*, α, by Melin and were later shown to belong to the genus *Boletus*. In the Gabelmykorrhiza the fungi isolated were termed *Mycelium radicis sylvestris*, β, γ and δ, respectively, and apparently belonged to the Hymenomycetes, although their exact identity was not ascertained.

Owing to the poor growth of the mycelium in artificial culture the mycorrhiza of spruce proved to be more difficult of investigation. Only one form was isolated, *Mycelium radicis abietis*. This was possibly a *Boletus*.

Melin was also able to synthesise mycorrhiza. Pine and spruce seedlings were raised from sterilised seed and grown in small flasks in sand or humus cultures. The seedlings were able to thrive in the absence of fungi, and showed certain morphological differences in their structure, such as the absence of differentiation into short and long roots. Inoculation experiments showed that each of the forms termed by Melin *Mycelium radicis sylvestris*, β, γ and δ, for pine, and *Mycelium radicis abietis* for spruce, formed mycorrhiza. It was only in sand cultures that these synthetic mycorrhiza were formed, since sterilised humus possesses marked toxic properties towards these root fungi.

Melin has also studied the mycorrhiza of birch and aspen. The mycorrhiza proved to be of the ectendotrophic type described above. In birch, as a general rule, there was a mantle of ectotrophic mycelium and then a palissade layer of large radially elongated cells with intracellular infection, and an inner zone in which digestion occurred. The fungi responsible for this association were *Boletus scaber* and *B. rufus* for birch and aspen, while *Tricholoma flavobrunneum* and *Aminita muscaria* also formed mycorrhiza with birch.

The mycorrhizal relations of larch did not meet with the success of the rest of Melin's work. He concluded that *Boletus edulis* formed an obligate symbiotic relation with larch. This view has been rejected by Hammerlund, and the subject requires further investigation.

The physiological aspects of tree mycorrhiza have also been considered by Melin. In the first place, the fungi concerned are all saprophytes and showed marked stimulation in contact with the roots of the host plants. This result is attributed to the presence of exudates from the roots, especially phosphatides. Carbohydrates are also possibly obtained, especially glucose.

Melin gives a qualified assent to the view first advanced by Stahl that the fungus partner of the association supplies the green plant with mineral salts. It is true that the roots can take up mineral salts whether they be infected or not, but the great poverty of mineral salts in the superficial layers of raw humus may give the green plant a greater advantage for the absorption of these substances when infection of the roots has taken place.

It was found that the endophyte in artificial media could utilise nucleic acids and other organic compounds, and in pure culture pine seedlings grown in the presence of the endophyte did not show the marked symptoms of nitrogen starvation compared with the controls—seedlings grown in the absence of endophyte. It is a well-known fact that woodland soils are markedly deficient in nitrates, and in acid humus soils plants with mycorrhiza would be at a great advantage, since they would be able to utilise the complex nitrogenous substances that are present through the medium of their fungus partners.

The Physiological Rôles of Endophyte and Host

The question arises : What is the precise *rôle* of endophyte and host in their physiological relations one to the other. It is obvious from what has been said in the preceding pages that these relations are probably diverse in the extreme. The older view was that there was some kind of mutual benefit between the partners of this arrangement. Actually there is a fine balance of adjustment between endophyte and host. Rayner found that if her cultures

of *Phoma* were vigorous and the seedling inclined to be weakly, then the fungus frequently consumed the seedling; Knudson obtained the same results with his orchid cultures (although this is denied by Ramsbottom), and concluded that the endophyte was definitely a parasite in nature, while Kusano discovered that in many cases *Armillaria* consumed and destroyed the tubers of *Gastrodia*. It is therefore possible that the mycorrhizal habit began by a parasitic attack of fungus upon green plant, and that this was at first repelled and later controlled to the advantage of the higher plant.

If the fungus be controlled, in what way does it benefit the higher plant ? In the very clear case of the Leguminosæ the green plant is supplied with organic nitrogen. It was natural, therefore, that the same suggestion should be made for endophytic mycorrhiza. Unfortunately, until recent years the evidence on this point has been highly conflicting. As far back as 1907, Ternetz made some very careful estimations on the nitrogen-fixing powers of *Phoma*, and recorded the fact that this fungus was able to fix free nitrogen from the atmosphere. Exact determinations by Nielson Jones and Llewellyn Smith have confirmed this work. Using the closed chamber apparatus devised by Blackman and Bolas, they showed that *Phoma radicis*, the endophyte of *Calluna*, is definitely able to assimilate atmospheric nitrogen. The use of an apparatus entirely closed to the external air, eliminated errors due to the presence of ammonia and other gaseous nitrogen compounds, the nitrogen used for this work being obtained electrolytically. The amount of nitrogen fixed was very small, and was of the order of 0·00095 gm. for every 0·345 gm. of glucose used.

In the case of the Orchidaceæ there is at present no definite evidence that the endophyte is capable of fixing molecular nitrogen. Wolff has made the claim that the endophyte he was able to isolate in pure culture from the roots of *Neottia* was able to assimilate free nitrogen, but this work requires reinvestigation, more especially after Knudson's observations. The views of the latter, and also those of Bernard, have been briefly discussed (see above). The *rôle* of the fungus in the Orchidaceæ would seem

to be that of hydrolyser of carbohydrates for the germinating seedling with its poor supply of food reserve. But even this result does not explain the presence of the endophyte in the roots after germination has been successfully accomplished. The fact that the hyphæ are consumed is significant, and it is equally significant that the endophyte is not allowed ingress to the stele. But whether Bernard's original view that the orchid suffers from a benign cryptogamic disease be correct or not, or whether the fungus has some definite physiological *rôle* in the metabolism of the higher plant has still to be shown.

Families of flowering plants which possess mycorrhiza show a gradual trend towards the saprophytic state. This is clear in the Orchidaceæ; it is equally clear in the Ericales, in which the Pyrolaceæ show a very marked series of forms which exhibit more and more strongly the saprophytic habit. It may well be that the first stage of the mycorrhizal habit was to make the seed dependent on the presence of the fungus for successful germination, and that later the fungus relieved the higher plant of some of its metabolic functions. If this be the case, then it would seem that the photosynthetic mechanism has been tampered with and the fungus supplies the higher plant with organic food. The evidence accumulated by McLennan seems to be very clear on this point. In *Lolium* there would appear to be little doubt that the function of the endophyte is to supply fat or oil.

Theory of Double Infection. Peyronel has advanced the view that two different fungi are involved in mycorrhizal infection. Peyronel's own work was mainly concerned with wheat, and he arrived at the conclusion that two different types of mycelium were present in the same cells and showed similar structural features. Later, however, he modified these views. Taking the case of the Orchidaceæ first, we have here a member of the Eumycetes as the endophyte; in the rest of the flowering plants the endophyte shows characteristics which point to it being a member of the Phycomycetes. He therefore regarded infection in endophytic mycorrhiza as being of a composite character. One of these, usually less well developed than the other, is of the *Rhizoctonia* type characteristic of the orchids; the

other shows features characteristic of the Phycomycetes. The mycelium of the latter has a wide distribution in damp humus soils and forms an investment round the roots of plants growing in such situations. It lives saprophytically on the dead tissues present in this substratum and forms vesicles rather similar in appearance to arbuscules. In the majority of plants Peyronel would consider that the following stages are passed through in fungus infection. The mycelium possessing arbuscules and vesicles first of all makes an entrance and develops chiefly in the outer cortex, where it produces arbuscules and behaves generally like a saprophyte or partial parasite. At a later stage a second invasion takes place by a mycelium of the orchid type of fungus. Peyronel has been able to isolate from a number of different species of flowering plants, such as *Solanum tuberosum*, *Nicotiana tabacum*, *Circæa alpina*, *Daucus carota*, *Beta vulgaris*, *Hordeum vulgare*, *Zea mais*, and others, fungi showing characters similar to the *Rhizoctonia* of orchids. He has not up to the present been able to isolate the endophytes belonging to the second group.

The problem of the second type of mycorrhiza, *i.e.*, ectotrophic mycorrhiza, has always led to a good deal of controversy. The early workers, *e.g.*, Tulasne and others, regarded the relation as one of simple parasitism. This view was later modified, and it was thought that the higher plant obtained some kind of benefit from the association, and the view came into prominence that the green plant did not draw mineral salts directly from the soil, but was aided in the process by the mycelial weft surrounding its roots. A still later modification of this view was that the fungus did not necessarily nourish the roots, but drew its supplies from the humus of the soil and passed on a portion of this supply to the higher plant. Another hypothesis that figures largely in the literature was that originally put forward by Stahl that mineral nutrients were passed on to the green plant by the fungus and deficiencies in transpiration were remedied. The recent work of Melin has added very materially to our knowledge of this part of the subject. This investigator's results have already been discussed under Tree Mycorrhiza, and will not be further considered here.

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ALGÆ

PHÆOPHYCEÆ

Introduction—Somatic Organisation of the Phæophyceæ—Classification — Ectocarpales — Sphacelariales — Dictyosiphonaceæ and Scytosiphonaceæ — Laminariales — Taxonomic Position of Chorda and Sacchoriza—Dictyotales—Fucales and the Alternation of Generations in the Phæophyceæ.

Introduction

THE whole subject of Freshwater Algæ has so recently been reviewed by Professor Fritsch in his revision of West's *Treatise on the British Freshwater Algæ* that little can be added here to that part of the subject. On the other hand, a number of investigations have appeared on the Brown (Phæophyceæ) and Red (Florideæ) Algæ, especially from the Continent, and on this account it will be more profitable to give a short description of the more important of these investigations. The present chapter is concerned with the Phæophyceæ, while the Florideæ will be treated in Chapter VIII.

Somatic Organisation of the Phæophyceæ

An excellent summary has been published by Dr. Church on the somatic organisation of the Phæophyceæ. Only a few points can be given from this memoir, and the original should be consulted for fuller details of the subject, although it should be stated, in parenthesis, that the book makes none too easy reading, owing to the author's somewhat condensed style and plentiful use of technical terms. In this connection the reader should also read Church's other memoir, *Thallassiophyta and the Subaerial Transmigration*, in which the evolution of a

Land Flora is dealt with from a new angle and with considerable vigour and freshness of outlook.

In his memoir (*Somatic Organisation of the Phæophyceæ*) Church gives a classification of the plant-forms in the Phæophyceæ in order of increasing complexity and efficiency of structure, and the relation of various structures to the factors of environment is discussed in detail. A short summary is given below of the series :—

1. *Ectocarpoid Forms.* The examples chosen are *Ectocarpus* and *Pylaiella.* The plants in this group are composed of much-branched uniseriate filaments. The chief advantage to be derived from this type of structure is that every cell is fully exposed to the external medium, but it also has the disadvantage of inability to withstand heavy seas and prevents the attainment of great size. *Sphacelaria* is also placed in this group. It differs, however, from *Ectocarpus* and *Pylaiella* in that intercalary growth is replaced by apical growth from a single large apical cell and the multiseptation of its products. This arrangement gives rise to increased strength, but nevertheless the plants remain small. Increased cortication in the higher members allows of cells in the older axes to receive less light, and these are no longer in direct contact with the external medium. *Cladostephus* is an advance on *Sphacelaria,* and shows further outgrowths from the primary cortex and develops a secondary cortex or " mantle," which materially adds to its mechanical strength.

2. *Cable Type.* *Mesogloia* may be taken as an example of this type of arrangement. Axial strands of large cells or filaments, which are frequently strengthened by interwoven hyphal growths, are present. The whole is invested with tufts of branches radially arranged like a palisade, and these are embedded in a matrix of mucilage. These tufts of branches (" ultimate ramuli ") bear the sporangia, and are also the photosynthetic tissue. *Chordaria* is a more advanced member of this group from the point of view of efficiency and condensation.

3. *Multiseptate Cable Type.* *Chorda* is taken as the chief example for this type of structure by Church. It is considered to extend longitudinally to the limit of mechanical cohesion and

hapteron-system, and individuals may attain as much as 40 feet in length in quiet waters, while a filamentous type only reaches a little more than a foot in range. Church also points out that it occupies the least area of the substratum, and thereby allows of "indefinite gregarious association."

4. *Corticated Type.* This group includes *Desmarestia, Arthrocladia, Stilophora* and *Spermatochnus.* In *Desmarestia* an axis is present consisting of branching uniseriate filaments with trichothallic growth, but this is obscured by the development of much pseudo-parenchyma, due to the growth of descending ramuli. This bulky mass of tissue may show a certain amount of differentiation, and the success of the strengthening device allows of the plants to attain a length of from 4 to 6 feet. During the summer the plants bear delicate branches in groups, and these are shed before winter, an apparently seasonal adaptation.

5. *Parenchymatous Type.* Laminate forms (*Punctaria*) and tubulate forms (*Asperococcus*) are included here. The parenchymatous type of structure is derivable from a *Chorda*-like type by the suppression of external ramuli, localisation and immersion of the sori and immersion of the sporangia in the parenchymatous thallus. According to Church, localisation of the sorus is always to be taken as the indication of a preceding condition of diffused production of similar ramalia-systems.

6. *Improved Parenchymatous Types.* The culminating members of this group are the Laminariaceæ and Fucaceæ, which present a large variety of different forms and show a high differentiation of members and elaboration of tissue-systems which are far in advance of any other group in the Thallophyta. For a full description of these forms the original should be consulted.

The function of the long hyaline hairs, which are characteristic of the great majority of the Phæophyceæ, has led to a good deal of conjecture from time to time, and the matter has been rediscussed by Professor Lloyd Williams in his Presidential Address to Section K of the British Association at Southampton. He has pointed out that the following suggestions have been made with regard to their use :—

(*a*) That they respire and absorb nutritive material.

(*b*) That they serve as shock-absorbers and prevent injury to the plant from friction.

(*c*) That they protect against intense illumination.

(*d*) That they protect against epiphytes. This is hardly true, for *Chorda* often bears luxuriant ectocarpoid vegetation.

(*e*) Church describes them (but with some hesitation) as mucilage organs, and calls them " mucilage hairs."

Lloyd Williams makes the suggestion that in muddy water these hairs effectively prevent the accumulation of sand and silt from settling on the thallus. He found, for example, when collecting *Dictyota* on oyster banks in the Menai Straits, that passing steamers sent waves that churned up the mud, which afterwards seemed to cover the *Dictyota* plants. A slight movement of the water, however, shook off the dirt, which was then seen to be caught and suspended in the web of hairs. Nevertheless, Lloyd Williams questions whether this feature be a primary function of the hairs.

The Phæophyceæ within recent years have been the centre of a number of particularly interesting researches on their methods of reproduction. Reproduction in this group varies from simple isogamy to pronounced oogamy, and in the higher forms there is a well-marked morphological alternation of generations.

The group is a remarkable one from several points of view. It forms the present dominant flora of the sea, and the higher members are the most highly differentiated, as regards their somatic construction, of all the Thallophyta. In form they range from simple discs of tissue through filamentous forms to a full differentiation of tissue systems. In some of the Laminariales, for example, the presence of true sieve-tubes have been described. In geographical distribution they range from the Arctic to the Antarctic shores.

Classification

The incompleteness of our knowledge of the reproductive processes in these forms until within recent years has made the classification of the group very empirical. Oltmanns, in his second edition of *Morphologie und Biologie der Algen* (*Zweiter*

Band), recognises seven orders : (1) Ectocarpales, (2) Sphacelariales, (3) Cutleriales, (4) Laminariales, (5) Tilopteridales, (6) Dictyotales, and (7) Fucales.

Kylin grouped (1), (2) and (3) into the single complex—the Phæosporeæ—and suggested that it contained the more primitive forms of the Phæophyceæ, from which two general lines of evolution have emerged : (*a*) towards the Fucales, and (*b*) towards the Laminariales, with a subsidiary branch leading to the Dictyotales and Tilopteridales :—

Laminariales

Dictyotales

Tilopteridales

Fucales

Phæosporeæ
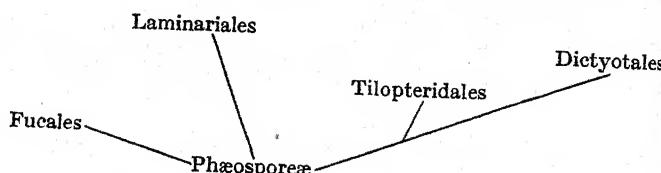
The taxonomic position of the Laminariales until the investigations of Sauvageau and Kylin was curious, inasmuch as this order of plants is possessed of a high somatic organisation of tissue systems, with true sieve-tubes in some cases, and yet, as regards their method of reproduction, only asexual zoospores were known. The discovery by Sauvageau of the gametophytic generation of these plants has, however, cleared away former difficulties, and they have now been moved to a position which competes with that of the Fucales.

Ectocarpales

Oltmanns recognises thirteen families in this order, but both Sauvageau and Yendo have shown that in two at least of these families (Dictyosiphonaceæ and Scytosiphonaceæ) there is a well-marked morphological alternation of generations with dominant sporophyte and microscopic gametophyte. More extensive investigations will probably reveal that other members of this order also possess two generations, so that further subdivision in the future may become necessary.

In the Ectocarpaceæ, which is usually held to be the most primitive family of the order, Miss Knight has found that the

methods of reproduction are more complex than was originally supposed. The thallus here is built up of ramifying filaments and the plants are often epiphytic on the larger Browns. Unilocular and multilocular sporangia are present. Till recently it was usually considered that asexual zoospores were contained in the unilocular sporangia, whereas the multilocular sporangia gave rise to the gametes. Miss Knight, however, has shown that for *Ectocarpus siliculosis* growing in British waters the reverse is the true state of affairs. She has investigated two genera in this family : *Pylaiella* and *Ectocarpus*, whose life-histories will now be discussed.

Pylaiella. The main difference between *Pylaiella* and *Ectocarpus* lies in the fact that the former possesses intercalary sporangia, whereas in the latter they are either lateral or terminal in position.

Pylaiella can undergo a complete cycle of change in form during its growth period, largely owing to the fact that truncations occur and the filaments break at the position of the intercalary sporangia. *Pylaiella* is an epiphyte on the larger Browns, and it has been ascertained by Knight that the host plant in early spring is *Ascophyllum nodosum*. The epiphyte makes its appearance between the main thallus and fruiting branches of the host. Later invasion spreads to *Fucus vesiculosis*, so that a second wave of invasion is generated in the early summer with *Fucus* as the host plant. The locus of attachment here is the mucilage hairs of the conceptacles. In late summer a third wave of invasion is generated, with *Fucus serratus* as host, and the locus of attachment is now the bushy mucilage hairs of the conceptacles.

Unilocular and multilocular sporangia are present, and Knight has now shown that a definite if somewhat fluctuating alternation of generations is present. There are two kinds of plants— haploid and diploid—the haploid bear multilocular sporangia, giving rise to zoids which fuse in pairs to form zygotes, and therefore are the true gametes, and finally diploid plants are produced. The diploid plants bear both multilocular and unilocular sporangia. The unilocular sporangia contain zoospores which bear the haploid number of chromosomes and give rise to

haploid plants once more, whereas the multilocular sporangia form neutral swarm spores carrying the diploid number of chromosomes, which later develop into diploid plants. Diploid plants can in this way be produced for a number of generations.

According to Knight, the cycle of these plants in their natural habitat is as follows : in the early spring the liberated zoospores give rise to haploid plants attached to *Ascophyllum nodosum*. The latter produce haploid gametes in multilocular gametangia which fuse in pairs and yield diploid plants. These latter now reproduce themselves by means of diploid neutral swarm spores, whose development leads to a long succession of diploid plants confined to *Ascophyllum* in the early summer, but gradually spreading to *Fucus vesiculosis*, and finally to *F. serratus*. On the latter, the cycle of development is completed with the formation of the haploid zoospores. The life-cycle is shown diagrammatically below :—

Life-cycle of *Pylaiella littoralis.*
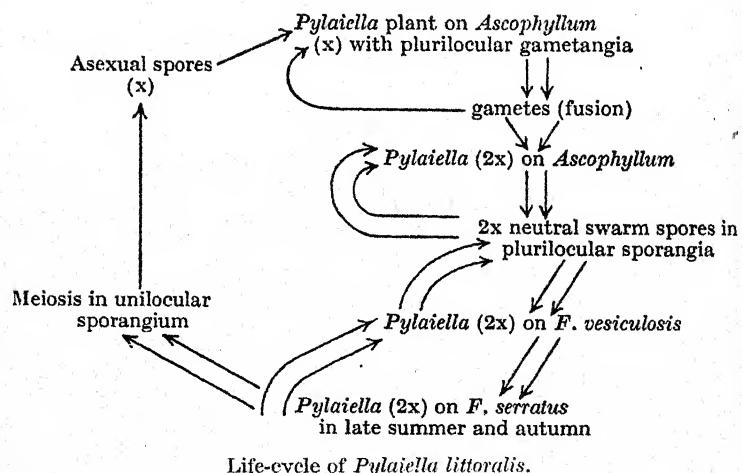
Knight is in agreement with Kuckuck that *Pylaiella* is a primitive form. If this be the case, it is of considerable interest to observe that the rather fluctuating type of alternation of generations exhibited by *Pylaiella* has become completely

stabilised in the Dictyotales. The parthenogenesis that has been observed in the genus is therefore not to be explained as a degradation of potential gametes, but rather as a primitive type of reproduction. Sexual reproduction by the fusion of isogamous gametes is considered to be of secondary origin, and finally, the reduction in chromosome number is postponed through many generations.

Ectocarpus. In *Ectocarpus* itself it has usually been considered that the products of the multilocular sporangia are gametes which fuse in pairs, while the unilocular sporangia give rise to asexual zoospores which germinate directly to produce fresh plants, and is a rapid method of propagation. The situation is, however, more complex than was originally supposed.

Miss Knight has investigated *Ectocarpus siliculosis* growing at Port Erin and also forms growing in Naples. This plant is a summer annual, but in quiet conditions has been known to persist to late autumn. Fucosan is produced in large amounts in the cells, and is usually arranged in glistening droplets round the nucleus in the central vacuole.

The various stages in sporangium development are associated with rhythmic changes in the supply of stored food material. At certain stages of development fucosan is present, and gives a strong reaction with vanillin and hydrochloric acid. A little later this may disappear and make its reappearance at a still later stage. Both unilocular and multilocular sporangia are present. The latter arise as polar sp. →2 off by an oblique wall from a cell of the filament. Increase in length occurs and cell partition is followed by cross-walls at right angles to the longer axis. Two cells are thus established, and further division leads to the formation of a short, stout filament. A definite zone of intercalary growth makes its appearance at the base of the filament, and the mature sporangium may consist of as many as 600, approximately, cubical loculi. The zoids are typically Phæophycean in shape. They are biciliate, pear-shaped bodies, with a central nucleus and two disc-shaped chromatophores, two pyrenoids and a conspicuous eye-spot. The unilocular sporangia are not of such common occurrence as the plurilocular organs. They are ovoid or elliptical

in outline, seated on a short stalk, or may even be sessile. The zoids are larger than those produced in the multilocular structures.

It will be necessary to discuss the results obtained from the Port Erin and Naples plants separately, since they differ in their behaviour in a number of important particulars. The plants were grown in artificial culture, and at Port Erin it was found that the earliest (from May onwards) bore plurilocular sporangia. Towards the end of August unilocular structures began to make their appearance on the older plants, but plurilocular sporangia were also present. The number of chromosomes in the somatic cells and in the zoids from the plurilocular sporangia was found to be 16,

Plurilocular sp. $\longrightarrow$ Zoids $\longrightarrow$ New Plant
(2x)   (2x)   (2x)
                      (asexual)

E. siliculosis
(2x)

Unilocular sp. $\longrightarrow$ Zoids $\longrightarrow$ Zygotes (fu $\rightarrow$ New Plant
(x)   (x)   (2x)   (2x)
                    (gametes)

Life-cycle of Ectocarpus siliculosis
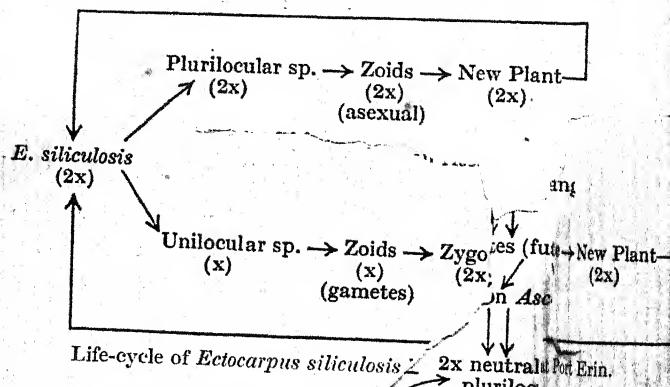
and meiosis was absent in this type

in the unilocular sporangium, but the first two divisions in the other, and, at first bore 16 chromosomes, and the zoids were found to be haploid. The plant body in the Port Erin species is therefore diploid, and the normal method of rapid propagation is by means of diploid zoospores. The unilocular sporangia arise in the autumn, release haploid gametes.

Examination of other species of Ectocarpus, e.g., E. fasiculosis, E. tomentosis, E. penicillatus, E. glutinus and Isthmoplea sphærophora, all showed that the plurilocular sporangia contained 16 chromosomes, i.e., were diploid, whereas in the last three species

the products of the unilocular sporangia were haploid (8 chromosomes).

Direct observation showed that the zoids from the plurilocular sporangia settled down and germinated directly into new plants, whereas copulation occurred between zoids from the unilocular structures. The life-cycle of the Port Erin plants is shown diagrammatically on page 194.

In the Naples plants, on the other hand, the situation was found to be very different and more complex in nature. Here the somatic tissues bore the haploid number of chromosomes, *i.e.*, 8, yet morphologically the plants were similar to the Port Erin species. The zoids from the plurilocular sporangia fused in pairs, and no unilocular sporangia could be found. Some years ago Berthold stated that unilocular sporangia are to be found on young plants raised as germlings in culture vessels. Thus, in the absence of direct cytological evidence, there are several alternatives possible in the life-history of the Naples plants, differing according to the point at which meiosis may occur in the life-cycle :—
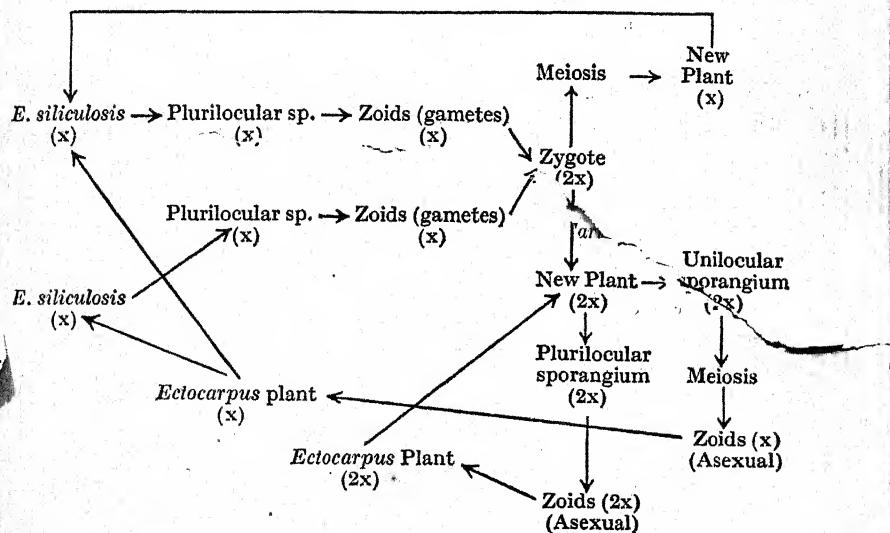
Life-cycle of *Ectocarpus siliculosis* living at Naples.

The question arises here whether environmental conditions affect the anomalous distribution of haploid and diploid thalli. In British waters the plants last from March or April till late autumn. In Naples they are produced at an earlier date (February), but die off in the heat of summer. It is possible that distribution is in some way affected by the temperature factor.

Ectocarpus Padinæ. Sauvageau (1920) has described a curious situation in this species. *E. Padinæ* is a parasitic Brown on the thallus of *Padina Pavonia*, and possesses three different types of plurilocular organ: "megasporangia," "meiosporangia" and "antheridia." The mega- and meiosporangia release zoids which germinate without fusion, *i.e.*, they behave like asexual zoospores. The spermatozoids, on the other hand, produced from the antheridia are apparently functionless. No unilocular organs of any kind have as yet been discovered.

Sphacelariales

This order includes three families, Sphacelariaceæ, Cladostephaceæ and Stypocaulaceæ. The thallus shows cortication, and growth in length is by means of an apical cell, and is not intercalary as in the Ectocarpales.

The life-history and cytology of *Sphacelaria bipinnata* (Sauv.) has been investigated by Clint. The plant occurs on *Halidrys siliquosa* on the Manx and Welsh coasts, but in more southerly regions *Cystoceira fibrosa* is the host plant.

The young plants of *S. bipinnata* are to be found on the fronds of *H. siliquosa* in February and March at a time when *Halidrys* starts into active growth once more. But it can only be seen at this period by microscopic examination.

The host tissues are penetrated to a considerable extent (Fig. 44), and it would appear that *S. bipinnata* is partially parasitic, since sections treated with vanillin and hydrochloric acid show a pink coloration in all parts of *Halidrys*, with the exception of the regions immediately surrounding the penetrating filaments.

Both unilocular and multilocular sporangia are present. Contrary to the views of Sauvageau, the former are the gametangia; the latter function as zoosporangia.

The unilocular sporangia are supported on a unicellular stalk. They are at first oval, but later become spherical and arise in the same way as lateral branches. A stalk-cell is separated from the sporangium rudiment and the latter is at first uninucleate. The mother cell of the sporangium now enlarges considerably. Two nuclear divisions occur and constitute a meiotic phase and the chromosome n u m b e r is halved. Further nuclear divisions follow and are at first confined to the periphery, but later move to the centre. The chromatophores also divide at the same time. The cytoplasm separates into rounded masses and each mass contains a single nucleus and four chromatophores and becomes the gamete. S p o r a n g i a l dehiscence is brought about by swelling; the wall dissolves locally and the gametes are liberated through an apical pore.

The plurilocular sporangia also occur laterally on unicellular stalks and are cylindrical in shape. In their formation a stalk-cell

Fig. 44.—*Sphacelaria bipinnata* on *Halidrys siliquosa*, showing penetration of host tissues. (After Clint, *Publ. Hartley Botanical Lab.*)

is separated from a sporangium rudiment. The nucleus of the rudiment undergoes division, the spindle being parallel to the long axis of the cell.

The products of the unilocular structure are undoubtedly gametes. The zoids are released in a mass surrounded by a gelatinous envelope. This envelope dissolves and the gametes are set free and swim about freely. They unite in masses of 2, 3, 4 and 5, and the individuality of each zoid is lost.

The zoids from the plurilocular sporangia emerge singly, and were never observed to fuse, and in size were approximately half that of the gametes from the unilocular gametangia.

In *Stypocaulon scoparium*, with the exception of a single plant described by Sauvageau, antheridia are unknown, and unilocular sporangia are the only reproductive organs known at present. The preliminary stages of division in the unilocular sporangia have been described by Higgins. The chromosome number in the somatic cells is 32, and it was found that the prophase of the first division in the unilocular sporangium was typically heterotypic in nature. Further confirmation of this was found in the number of chromosomes in the nuclei during the subsequent stages in the sporangium, *i.e.*, 16 were present. As antheridia appear to be absent or very rare, it would be interesting to know the subsequent fate of the products of the unilocular sporangium.

Dictyosiphonaceæ and Scytosiphonaceæ

Both these families are included by Oltmanns in the Ectocarpales. But since the discovery in 1917 by Sauvageau that in *Dictyosiphon fœniculaceus* there is a morphological alternation of generations, and in 1919 by Yendo that a similar state of affairs is present in *Phyllitis fascia* of the Scytosiphonaceæ, their toxonomic position becomes a matter of considerable difficulty. In fact, Taylor has gone so far as to elevate the Dictysiphonaceæ to ordinal rank—the Dictyosiphonales. Logically, the Scytosiphonaceæ should be treated in the same way. It will be convenient to consider these two families together at this stage.

Dictyosiphonaceæ. It has been known for a number of years that unilocular sporangia were the only reproductive organs of this family, and it was suggested as far back as 1876 by Areschoug that the zoospores could on occasion play the part of gametes. In 1917 Sauvageau showed that the zoospores develop to give a gametophyte. The zoospores are pear-shaped bodies with an eye-spot and possess a single chromatophore. They come to rest, withdraw their cilia and germinate to give a prothallus. This structure at maturity is about a millimetre in diameter and rather resembles *Streblonema* in general appearance. Cylindrical

multilocular gametangia are developed on the prothallus, each with 2 to 12 loculi. Each loculus gives·rise to a single gamete, which escapes by means of a terminal pore. Conjugation was not observed, but undoubtedly takes place, since rounded bodies were observed with two nuclei and two chromatophores, and these germinated to give the essential thallus of *Dictyosiphon*.

Scytosiphonaceæ. As in the Dictyosiphonaceæ, no gametangia had previously been observed in the Scytosiphonaceæ. It was noticed by Yendo in *Phyllitis fascia* that the zoospores are strongly phototactic and of the usual Phæophycean type, with chromatophores, eye-spot and unequal cilia. They come to rest and form themselves into spherical masses and remain in this condition for a week. A short process is now thrust out at one side. Nucleus and chromatophore divide and a cell wall is laid down. Further division leads to the production of a protonemal-like structure. A wart-like process is formed on the initial cell and later develops into a hair.

The cells of the filament now undergo great changes, and one or more swell up greatly and show dense contents and become mucilaginous. These are the antheridia. In other filaments similar changes also occur, and the chromatophores become more intense in colour, and frequently a series of swollen cells are formed. These are the oogonia.

Laminariales

Two families are at present recognised in this group—the Chordaceæ (with one genus, *Chorda*) and the Laminariaceæ. Further subdivision of the order is probably necessary, and Lloyd Williams has suggested that *Sacchoriza* should now be elevated to family rank.

There is a well-marked morphological alternation of generations in the group. The conspicuous generation is the sporophytic one. These produce zoosporangia which are contained in elongated sac-like organs (Fig. 45, 1). The sporangia are produced in more or less well-marked sori, which are localised in character and are mixed with paraphyses. *Chorda* is exceptional in this connection, and the sporangia occur over the whole surface (Fig. 45, 2). In

Alaria and its allies (*Hirome*, *Undaria* and *Pterygophora*) they are restricted to special sporophylls. In *Postelsia* and *Macrocystis* the sporangia are borne on longitudinal furrows in the fronds. The paraphyses are non-septate structures, and, with the exception of *Sacchoriza* and *Chorda*, possess a special hyaline appendage at the tip.

The zoospores from the zoosporangia germinate to give a protonemal-like prothallus, which is the haploid or gametophytic
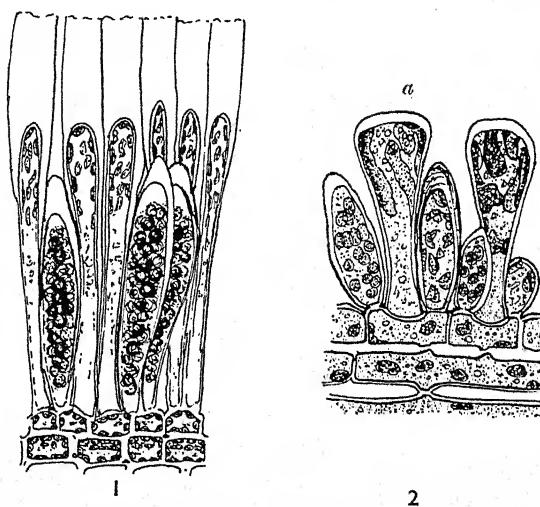
FIG. 45.—(1). Sporangia of *Laminaria saccharina*. Note the presence of paraphyses with hyaline appendages (Schleimkappe). (2). Sporangia of *Chorda filum*. Note the absence of paraphyses with hyaline appendages and the presence of assimilating cells (*a*). (From Oltmanns, *Morphologie u. Biologie der Algen*.)

generation. Up to 1915 nothing was known of the sexual reproduction of these forms, and it is only since that date that the solution of the problem has been found, largely owing to the labours of Sauvageau and Kylin.

In 1910 Drew described the zoospores in *Laminaria digitata* and *L. saccharina* as fusing in pairs. Later (1912) Lloyd Williams pointed out the inherent improbability of such a state of affairs, since at that time it had been established in other groups of the

Phæophyceæ that the products of the unilocular sporangia were the asexual form of reproduction. In 1915 Sauvageau announced his discovery of the gametophyte of *Sacchoriza*. Prior to this discovery, Lloyd Williams had actually seen the gametophyte generation of some of these forms, but was unable to interpret them.

Sauvageau showed for *Sacchoriza* that the zoospores come to rest, round themselves off and later become pear-shaped—the length greatly exceeding the diameter. The contents of the zoospore pass to the narrower end, leaving the lower region empty. This is the female gametophyte. The male gametophyte is a rather more complicated structure, and is composed of several cells. On the male prothallus each antheridium gives rise to a single sperm with two lateral cilia.

The zoospores from a single sporangium were found to give rise to both male and female gametophytes. In a number of cases the sporangia did not liberate their zoids ; the walls became greatly swollen and the zoospores germinated into the two kinds of gametophytes while still enclosed within the sporangial wall.

A year later (1916) Sauvageau discovered the gametophytes of *Laminaria flexicaulis* and *L. saccharina*. As in *Sacchoriza*, the zoospores come to rest, round themselves off and nucleus and chromatophore divide, and daughter halves pass into either end of an enlarged tube which has now been formed by the zoospore. A wall is laid down across, and an " *embryospore* " is cut off from the rest of the structure. Further development leads to the formation of a few rows of cells, and male and female prothalli are formed. As in *Sacchoriza*, each antheridium gives rise to a single sperm, which is released before the female gametophyte in the same culture has reached maturity. The female gametophyte is a more complex body than in *Sacchoriza*, and is composed of several cells, and any cell can give rise to an oogonium. Before fertilisation (which was not observed by Sauvageau) the oogonium swells, elongates and the oosphere emerges through a terminal aperture at the end of the protuberance.

Kylin has confirmed this work of Sauvageau for *Laminaria digitata*, and Lloyd Williams has discovered the presence of the

gametophytic generation in a species of *Laminaria* in this country.

Like Sauvageau, Lloyd Williams (1921) found that the zoospore
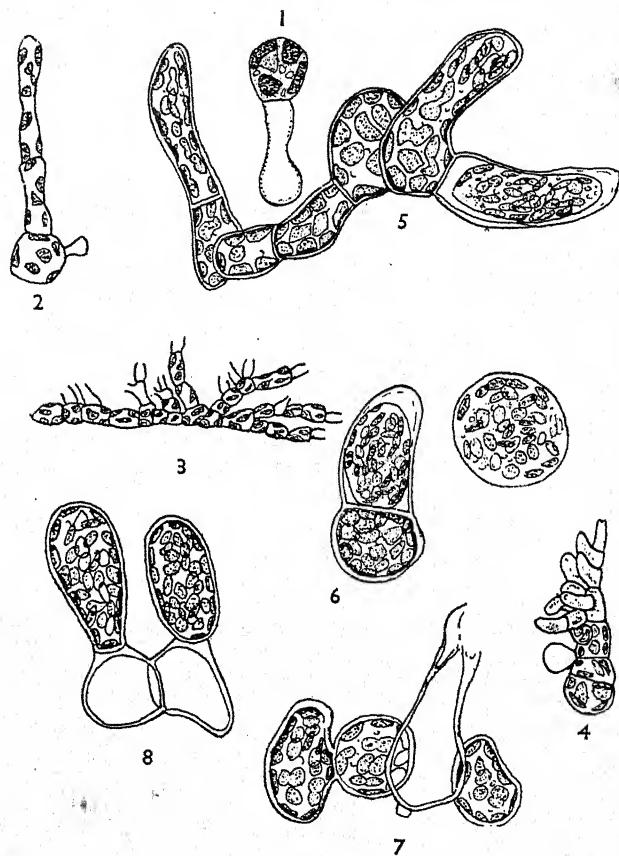
FIG. 46.—(1–3). Development of the gametophytes of *Laminaria digitata*. (4–8). Gametophyte and germination of *L. saccharina*. (From Oltmanns, *Morphologie u. Biologie der Algen*.)

came to rest and developed into a pear-shaped body. The greater part of the cytoplasm travelled into the narrower end, while the nucleus remained behind. The distil end then began to swell and the nucleus divided, one of the daughter nuclei migrating into the

newly formed enlargement. A wall was formed across and the gametophyte elongated slightly. The wall covering the narrower end became greatly thickened, and was later differentiated into three layers, the middle layer showing a lamillated appearance. A narrow fissure then appeared at the opening of the oogonium and the contents were pressed out. When the whole of the protoplast had emerged, the thick wall closed elastically, and the only evidences of the aperture were a faint line and a rim or collar, enclosing the base of the protoplast, which remained seated in a narrow cup.

In *Chorda* the gametophytic generation is a larger structure than in *Laminaria*. The protonemal-like strands branch again and again in a very irregular manner. The egg emerges in the same way as in *Laminaria*, but always remains enclosed within the inner wall of the sporangium. The wall of the oogonium swells slightly, the outer layers burst, and the contents, still attached to the extensible inner wall of the oogonium, pass out to the exterior.

Lloyd Williams (1921) has made some interesting observations on the fertilisation of the egg in *Chorda*. Fertilisation may not take place for some six months, and certainly does not occur at a shorter time than three months after the formation of the gametophyte. The oospheres, after their emergence from the oogonia, were observed to be surrounded by spermatozoids, and preparations were obtained showing two gametic nuclei within the newly fertilised eggs.

The Japanese genus, *Laminaria religiosa*, has been investigated by Ikari. As in the other species of *Laminaria*, the gametophytes were usually dioecious and only rarely monoecious. The antheridia were often formed in a continuous row, and in general appearance were very similar to the plurilocular sporangia of *Ectocarpus*. The oospheres escaped through a terminal aperture of the oogonium and remained seated in a funnel-shaped hollow formed by the neck of the oogonium.

A curious situation has been described for *Macrocystis pyrifera* by Delf and Levyn. The sporangia here occur in special sori on more or less specialised fertile fronds. The zoospores appear

to be of two sizes, and give rise to filaments of different lengths (Fig. 47). Many weeks later a young sporophyte could be seen attached to these structures.

Doubt has been cast as to whether these curious protonemal-like filaments obtain in the sea, and it has been suggested that they are possibly the products of artificial culture. This objection, however, is no longer valid, for Ikari has claimed to have seen the gametophytic generation of *Laminaria religiosa* in the natural
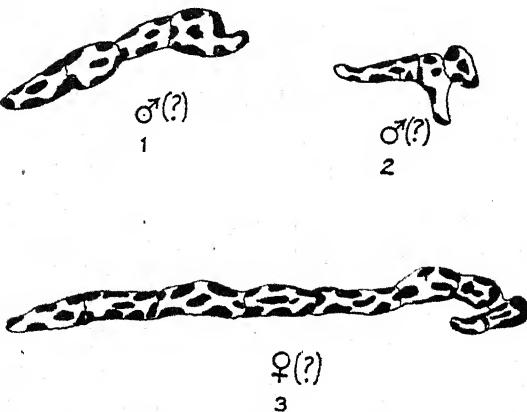
Fig. 47.—*Macrocystis pyrifera.* (1) and (2). Presumably male gametophytes. (3). Presumably female gametophyte. (After Delf & Levyn, *Anns. Bot.*)

habitat of this plant, while Lloyd Williams has observed the gametophytes of *Chorda* in the sea at Aberystwyth.

The Taxonomic Position of Chorda and Saccoriza. Till the discovery of the gametophytic generation of *Chorda*, its taxonomic position gave rise to a good deal of discussion. But both the histology as well as the method of reproduction, as they are now known to us, undoubtedly show it to be a true Laminarian. It possesses, however, a number of peculiarities which mark it out as being somewhat apart from the rest of the family. According to Church it is possibly a primitive type. It is a characteristic feature of the muddy reaches and gravel bottom of the sea in which the larger Laminarians and Fucoids cannot obtain a secure

footing.   In length it may reach as much as 40 feet as a slender whip-like thong, and in morphological features it is unlike the rest of the family.   Moreover, as Lloyd Williams has pointed out, the fact that the oosphere does not emerge from and remains attached to the inner wall of the oogonium, and that the mouth of the oogonium remains open, shows that *Chorda* is perhaps not so closely related to the other genera.   Three other facts have also to be considered : the peculiar form of the thallus, the fact that the sporangia are distributed over the whole surface, and, in spite of its energetic growth, its annual character.

The taxonomic position of *Sacchoriza* is also difficult.   In its internal structure it is so different to the other genera that Lloyd Williams (1925) has suggested that it should take its place as a separate family.   It is true that the method of reproduction is closely similar to that of the rest of the Laminarians, but in other features it is widely different.   The whole plant is unique.   The large basal bulb, the twist at the base of the large flat stipe, the curious furbelows and the strong fibre-like cells that are present, and the fact that the sporangia cover the base of the bulb, as well as the stipe and part of the lamina, the disappearance of the enormous lamina and stipe at the close of the growing season, and the continuation of spore production during winter on the persistent bulb are characteristics peculiar to *Sacchoriza* itself.

Dictyotales

There is a well-marked alternation of generation present here. The reproductive organs are of three kinds—male, female and asexual.   The female sexual cells consist of non-ciliated oospheres and the spermatozoids have but one cilium.   The asexual spores are non-motile and are known as tetraspores, being borne in groups of four in tetrasporangia.   Thanks to the labours of Lloyd Williams and others, the formation, development, and method of fertilisation are now well known and need not be discussed here. There are, however, a number of curious and obscure points in connection with the fate of the tetraspores and the development and distribution of the sexual plants that are still in need of investigation.

It was shown by Lloyd Williams for *Haliseris, Taonia* and *Dictyota* that the two divisions in the tetraspore mother-cell constitute a meiotic phase. More recently Carter has also shown for *Padina Pavonia* that tetraspore formation follows a reduction division in the tetraspore mother-cell. In *Padina*, and also in *Dictyota*, it is known that the tetraspores germinate to give sexual plants and the fertilised eggs to give tetrasporic plants. Nevertheless, *Taonia* and *Padina* growing in British waters are almost entirely tetrasporic. Similarly, *Dictyota dichotoma* obtained from Cardigan Bay is always asexual. *Padina Pavonia* is mainly to be found in the Mediterranean, and has but a limited distribution in these islands. On the coasts of Hampshire and Cornwall, Carter found an abundance of asexual plants, but no sexual forms. Yet the tetraspores germinate with extreme ease to give sexual plants. It was suggested by Church many years ago that the distribution of *Cutleria* and *Aglaozonia*, the gametophytic and sporophytic generations of the Cutleriaceæ respectively, was largely dependent on temperature. This suggestion, however, hardly applies to the Dictyotaceæ, since the gametophytic and sporophytic generations are morphologically identical.

It is extremely difficult from the facts to account for the succession of these plants on our coasts. The tetraspores are released in millions, and cultural experiments have shown that their germination gives rise to the sexual generation. Neither will parthenogenesis supply a reason. The unfertilised oosphere in the absence of the sperm merely undergoes a few segmentations and then dies. The Dictyotaceæ, unlike the Cutleriaceæ, appear to have lost the power of apogamous reproduction. At present there is no convincing explanation of this curious and interesting problem.

The Periodicity of Fruiting in the Dictyotaceæ. The fruiting of *Dictyota* has been investigated by Hoyt on the Jamaican and New Carolina coasts. It was shown some years ago by Lloyd Williams that *Dictyota dichotoma* at Bangor produces its sexual cells at fortnightly intervals during the summer season just prior to the neap tides, and the gametes are released two or three days after the succeeding spring tide. On the other hand, in October the

position is practically reversed, and the production of the crops
is much retarded. In Plymouth the sexual organs are also
produced at fortnightly intervals, which bear a definite relationship
to the tides, but the times differ from those prevailing at Bangor.
Similarly, it was shown by Lewis at Naples that the formation of
the sexual cells takes place at definite periods of fourteen days.
The rudiments appear at about the time of the neap tide, and the
mature gametes are released at the following neap tide. Hoyt
discovered that *Dictyota dentata* growing at Port Antonio and Port
Morant, on the Jamaican coast, also showed evidence of periodicity
in the formation of its sexual organs. The female plants from
Port Morant bore crops of two different ages, the younger of
these being at the corresponding stage of development to the sori
of the male plants collected at the same time, but the length of
time taken by the crops to mature is so long that there is no
perceptible development in them over a period of twenty-one days.
Hoyt also examined the question of periodicity over a number of
years at Beaufort in New Carolina during the summer season.
In this region the fruiting of the plants invariably occurred at the
spring tides of the full moon, quite regardless of a greater or lesser
set of spring tides of the lunar month. So clear was this relation-
ship of fruiting to the time of the full moon that it was possible to
accurately forecast the fruiting period from a calendar giving
the date of the full moon. The presence of a full moon was the
requisite factor, and the actual height of the tides did not affect
the situation. Even during October, when the weather was cold
and fruiting was generally retarded and irregular, no set of
conditions could induce fruiting to take place other than at the
time of a full moon. It is interesting to see how firmly impressed
is this periodicity upon the plants. Hoyt, for example, removed
specimens to the laboratory, and, although it is true a number
died, those that still retained living portions of thallus bore fruits
at the same periods as plants living under natural conditions.
The tetrasporic plants, on the other hand, show no such periodicity
as the sexual forms.

According to Hoyt, no single factor, or even group of
factors, will account for the curious rhythmic fruiting shown by

Dictyota. It is apparently independent of external conditions, such as temperature and height of the tide. Nevertheless the periodicity has become deeply impressed on the plants, and the rhythmic fruiting periods have been synchronised with rhythmic external conditions; in fact, so much is this the case that when the plants are removed to laboratory conditions away from the influence of their natural rhythmic habitat, the fruiting still synchronises to the conditions of their habitat.

Fucales and the Alternation of Generations in the Phæophyceæ

There has been little investigation of the Fucales within recent years. Williams, however, has described a curious life-history for the parasitic Fucoid, *Notheia anomala*, growing in Australian waters. The host plant is *Homosira Banksii*, when the latter is growing in tidal pools, so that the host is always covered with water, even at times of the lowest tides. *Notheia* is found to be absent when *Homosira* is growing in other positions.

The parasite grows out of the conceptacle of the host, and is most frequently to be discovered in the antheridial conceptacles, although it is also to be found in the oogonial to a certain extent. A hold-fast-like structure is produced in the cells of the host, although no definite haustorium is produced, as in many of the fungi. This absorptive organ consists of loose, rather elongated cells, arranged end to end, which gradually penetrate to the medulla of the host.

Notheia anomala produces conceptacles which are typically Fucoid in shape and which are monœcious. Up to the present, Williams has only been able to find oogonial conceptacles; the antheridial appear to be absent. Oogenesis conforms to the general conditions found in the Fucaceæ. The oogonia are developed from the walls of the conceptacle in the usual way by a papilla-like outgrowth. The nucleus divides and wall formation follows so that a stalk cell is separated from oogonial rudiment. The oogonium increases in size, and three nuclear divisions lead to the production of eight oospheres. Since antheridia are apparently absent, it would be of interest to know whether

mitosis in the oogonium leads to the reduction of chromosome number. Presumably, if antheridia be absent, the eggs must develop parthenogenetically.

The discovery of a fluctuating alternation of generations in *Pylaiella* and a morphological alternation in the Laminariales opens up anew the whole question of alternation of generations in the Phæophyceæ. The main discussion is, however, concerned with the situation presented by the Fucaceæ. According to Strasburger, *Fucus* is to be regarded as a sporophyte, and the so-called oogonia and antheridia are to be regarded as corresponding to the tetrasporangia of *Dictyota*; in other words, they are mega- and microsporangia respectively, and the gametophytic stages are limited to the few succeeding nuclear divisions, resulting in the formation of oospheres and sperms. Yamanouchi has taken a similar standpoint : " In *Fucus* the antheridial and oogonial initials may well be compared with the spore-mother cells of higher plants, and the four nuclei in these structures thus produced may be compared with microspores and megaspores, which in *Fucus* germinate at once within oogonium and antheridium and the gametophytic generation thus initiated undergoes only one mitosis in the oogonium and four in the antheridium." Lloyd Williams has also taken a similar view. The case of *Laminaria*, in which the gametophyte consists of a single cell separated from the zoospore by a single nuclear division, makes it easy to adopt the suggestion that the so-called oogonia and antheridia of *Fucus* are sporangia.

Church, on the other hand, has taken the view that *Fucus* is a gametophyte, and is thus comparable to an animal. There is only one method of reproduction in the Fucaceæ—by oospheres and spermatozoids. To Church " *Fucus* represents a perfectly straightforward story. It stands on its own merits. In older phraseology the soma is a gametophyte, because it actually is a plant (phyton) which reproduces gametes (and nothing else). To call it the converse to bolster up what is now an ancient speculative suggestion of Strasburger (1894) is the *reductio ad absurdum* of ill-advised theory."

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CHAPTER VIII

A L G Æ (*continued*)

FLORIDEÆ

Male Organs of the Florideæ—Bangiales—Nemalionales—Abnormalities in the Production of Reproductive Organs in the Floridéæ—Life-history and Taxonomic Position of Certain Selected Floridéæ.

A SHORT, but excellent, historical introduction to the Florideæ has been written by Dr. Church in the *Journal of Botany*, and those interested in this aspect of the group will find therein the gradual manner in which it came to be realised that the Red Algæ were a large and significant group of plants, with, in many cases, curious and elaborate life-histories. The account is also interesting from the author's views on the alternation of generations and its significance, which is fully dealt with by him.

The Male Organs of the Florideæ

A comprehensive investigation has been made by Grubb on the male organs of the Florideæ, and the following account is a summary of her more general conclusions.

The usual position of the antheridia is in superficial sori. In certain instances these may be scattered without order, as in *Rhodophyllis bifida* and *Cystoclonium purpurascens*, or the antheridia may be developed in acropetal succession, as in *Rhodymenia palmata* and *Chondrus crispus*. A series of transitions can be traced in the Delesseriaceæ from sori developed on the thallus to those on special leaflets, while in *Furcellaria fastigiata* the special structures take the form of a swollen apical "pod." Sori sunk in conceptacles are to be found in the Corallinaceæ, and possibly also in species of *Gracilaria*.

As a rule, the sexual plants of the Florideæ are diœcious, but the

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following among the Nemalionales have been reported to be monœcious in habit : *Nemalion multifidum*, *Batrachospermum*, *Helminthora*, *Helminthocladia* and *Bonnemaisonia asparagoides*.

In nearly every case the young antheridial plant is normal in appearance and resembles in every respect the young procarpial or tetrasporic thallus. In *Martensia fragilis* and *Cologlossa Leprieurii* it is smaller than the female and has a more ephemeral existence, and Dunn claims that the male plant of *Dumontia filiformis* dies after discharging its spermatia.

The antheridia in the Florideæ are invariably developed from a cell which has been variously termed by different investigators "cellule anthéridifère," "Spermatangienmutterzelle" or the "antheridial mother-cell." In the vast majority of cases these mother-cells are the terminal cells of branch systems. Exceptions to this rule are *Nemalion multifidium*, *Callithamnion roseum* and *Halurus equisetifolius*. This antheridial mother-cell is unbranched and gives rise, either terminally or subterminally, or even laterally, to antheridia.

Whatever be the number of nuclei in the vegetative cells, the mother-cells are always uninucleate and the nucleus in the resting stage lies near the base of the cell. The pigment in the cell also varies in amount in different species. The most common state is for the cell to show reduced pigmentation or rudimentary chromatophores, but in *Griffithsia Bornetiana*, *Kylinia rosulata* and *Dumontia filiformis* the mother-cells are said to be completely colourless, while in *Nemalion multifidum* and *Batrachospermum*, in which the mother-cell is homologous with a vegetative cell, the pigment is found in both in equal quantities.

The antheridia are developed alternately in no particular order or number in *Nemalion* and *Batrachospermum*. Among the higher Florideæ, however, their development has become more regular, and a definite number of primary antheridia are produced in a subterminal, rather than in a lateral, position, and ripen in orderly sequence around the upper surface of the mother-cell. The number of antheridia which arise from each mother-cell varies from two to four. The presence of four antheridia to a mother-cell is, however, of rare occurrence.

In the primary stage, antheridia develop as protuberances of the wall and protoplast of the mother-cell, and the daughter nucleus then formed by the division of the mother-cell nucleus passes into these slight projections. In shape, the young antheridium is either long and narrow, or round and swollen. When it is equal in length to the mother-cell, the contents are abstricted from below by a ring-like ingrowth of the surrounding elongated mother-cell wall, the constriction generally taking place at a level with the upper surface of the mother-cell, though in *Ceramium rubrum* it is above this level.

The antheridium now ripens with considerable rapidity; it swells in size and the cytoplasm becomes vacuolate and the nucleus takes up either an apical or central position. The wall shows considerable thickening and in a number of cases can be observed to consist of three layers. The thicker outer wall is generally more dense at the apex, the middle wall shows as a clear swollen central layer, and finally around the contents which will form the spermatium there is a narrow, highly refractive region. The mature spermatium is discharged through a split formed at the apex of the elastic surrounding wall, and through this narrow aperture the cytoplasm and nucleus slip out as a single spermatium.

There is still a good deal of doubt whether the spermatium is surrounded by a wall at the time of discharge, and various statements have been made on the matter from time to time. According to Grubb, in the fifteen cases examined by her, the spermatium when liberated appears to be surrounded by a delicate and highly refractive wall, and in some cases this wall appears to be present while the spermatium is still enclosed within the antheridium (*Polysiphonia fastigiata* and *Laurencia pinnatifida*).

In the development of the spermatium a uniform and characteristic series of nuclear changes takes place in the mother-cell as well as in the antheridium. The single nucleus of the mother-cell, which is, as a general rule, to be located at the base of the cell, appears in the resting condition to consist of two regions: a central denser region, and a colourless outer area. At the first signs of the formation of the spermatium the nucleus passes to the base of the small projection and there enters into the

early prophase stage, and finally passes through all the stages of mitosis. Of the two daughter nuclei formed in this manner, one returns to the base of the mother-cell, whereas the other enters the young antheridium. This mother-cell nucleus may repeat the process as frequently as eight times in the case of those species which form four primary and four secondary antheridia from each mother-cell.

The subsequent fate of the spermatial nucleus appears to vary in the lower and higher Florideæ. In *Nemalion* and *Batrachospermum* it is said to divide and give two daughter nuclei when the spermatium becomes attached to the trichogyne. In other genera, however, this division is known not to take place, but the spermatial nucleus while still in the prophase passes down the trichogyne and fuses with the carpogonial nucleus. It is possible that the marked difference in this respect between the behaviour of the lower and higher Florideæ points to what is now a single spermatium in the former group, as originally possessing the potentiality of becoming two, whereas in the higher group even this nuclear division has become suppressed, and the only remaining sign that it ever occurred is the condition of prophase in which the nucleus is found at the time of fertilisation.

Grubb has discussed the homologies of the antheridium in the Florideæ and compared it with similar structures in three different groups of plants. Among the Algæ, the only close resemblance is to be found in the Chlorophyceæ, and the similarity with *Coleochæte* has long been known and recognised. The antheridia in *Coleochæte* arise in position and manner almost exactly comparable to that found in the Nemalionales. But the comparison cannot be carried further, for the liberated spermatozoid is both naked and ciliated. The resemblance between the Florideæ and certain groups of the Fungi has also been commented on by different writers, and a number of parallels have been drawn between the oogonium (ascogonium) in the Ascomycetes and the carpogonium of the Florideæ, and in many species of Ascomycetes, especially among the Pyrenomycetes, spermatia are known. The spermatia of the Laboulbeniales offer a still better comparison, and in this group have been developed along two lines : exogenous

spermatia cut off as uninucleate, rod-shaped bodies from a specialised branch, and endogenous spermatia, which are constricted off, one after the other, from a mother-cell and covered by a thin protoplasmic membrane, and are extruded through a more or less elongated, flask-shaped neck. Comparisons, however, such as these throw but little light on the origin of the antheridial complex and only tend to emphasise the highly developed character of the male organs in the Florideæ.

In 1908 Svedelius published a classification of the Florideæ based upon the methods of spermatial production. The two criteria that were used by him for subdivision were based upon the structure of the antheridial mother-cell, and whether this resembled or differed from a vegetating cell, and also whether it were simple or branched. Gruvsions and the lights classification and rejected the second chase view. Kantuting in its place the variations in number and position of the antheridia. Her revised classification is as follows :—

A. Antheridial mother-cell not differing from vegetative cell either in form or contents (*Nemalion, Batrachospermum*).

B. Antheridial mother-cell differentiated from a vegetative cell.

1. Antheridia developed terminally on the mother-cell (*Martensia, Melobesia, Harveyella (Holmsella) pachyderma*).

2. Antheridia developed subterminally on the mother-cell.

(*a*) Mother-cell subtending two primary subterminal antheridia (*Delessaria sanguinea, Rhodymenia palmata, Chondrus crispus, Furcellaria fastigiata, Lemanea fluviatilis, Laurencia obtusa*).

(*b*) Mother-cell subtending two *or* three primary subterminal antheridia (*Scinaia furcellata, Cystoclonium purpurascens, Delessaria ruscifolia, D. alata, Lomentaria clavellosa, Plocamium coccineum, Polysiphonia nigrescens, Rhodophyllis bifida*).

(*c*) Mother-cell subtending three primary, subterminal antheridia (*Bonnemaisonia asparagoides, Polyides rotundus, Ceramium rubrum, Griffithsia corallina, G. Bornetiano, Nitophyllum Hilliæ, N. laceratum, Champia parvula, Dumontia filiformis, Callithamnion brachiatum, Rhodomela virgata, Polysiphonia fastigiata*).

(*d*) Mother-cell subtending four primary subterminal antheridia

(*Polysiphonia violacea, Chondria tenuissima, Laurencia pinnatifida, Callithamnion roseum, Halurus equisetifolius*).

It is evident that the position of the antheridia, as well as their number, varies greatly even among genera which from other grounds are regarded as being closely related. " Yet any intensive study of the male organs of this group cannot fail to impress the inquirer with a sense of the remarkable uniformity exhibited in the details of development and liberation in every case. . . . Similarly, no deviation has been observed so far from what has come to be regarded as the normal cytological behaviour in the antheridium." Taken as a whole, the course of events in the development of the male organs of this group seems to point to a probable common origin of, at any rate, the higher Flæ propr. trichogyne and fuses with the carpogonial nucle

Bangiales

In 1919 Yendo stated that the carpospores of *Porphyra tenera* germinated to give rise to male and female swarming gametes. Okamura, Ondo and Higashi, however, have failed to confirm this statement for *P. leucosticta*, and suggest that the " gametes " observed by Yendo were probably the zoospores of some Chytrid parasitising the carpospores.

The cytology and life-history of *Porphyra tenera* has now been investigated by Ishikawa. The method of nuclear division is interesting and peculiar, and assumes a type between true mitosis and amitosis. The nucleus in the somatic tissues of this plant is minute, and generally spherical in shape, and is situated as a rule near a radiating arm of the large, stellate chromatophore which occupies the cell.

At the time of division an achromatic space makes its appearance in the nucleus and is usually somewhat fusiform in shape. With further development of this achromatic space, the nucleus becomes elongated and spindle-shaped. The chromatic portion covering the achromatic core splits into three filaments by means of longitudinal fissures. As a rule, these fissures do not develop any further, with the result that the filaments are not completely separated from each other at either end and the unseparated portions generally pass into a fine process. The substance, or

substances, composing the achromatic region in the nucleus become mingled with the surrounding cytoplasm through these fissures, and eventually each filament becomes slowly thinner and finally breaks into two segments. These daughter segments withdraw to either pole of the nucleus, coalesce and form two new daughter nuclei. Ishikawa regards these chromatic filaments as chromosomes of primitive type. Nuclear division is now followed by division of the pyrenoid and chromatophore, and cross-walls are laid down to give two daughter cells.

The development of the spermatium was also followed. Repeated division of the vegetative cells in the peripheral portion of the thallus leads, in the first place, to the formation of antheridia. These antheridia are distinguished from the vegetative cells by their smaller dimensions and the lighter colour of their chromatophore in surface view. Each antheridial cell divides into two with the dividing plane parallel to the surface of the thallus. A second plane is now formed perpendicular to the first and cuts each cell into two equal daughter cells. A third dividing plane is laid down at right angles to the planes that have already been formed and the octant stage is reached. The nuclear divisions that precede cell division are of the same type as those described for the somatic tissues. Each cell of the octant also divides three times in the manner just described, with the result that it gives rise to a mass of eight cells. In this way each antheridium gives rise to sixty-four cells. The ultimate cells become spermatia, which are set free in succession. The mature spermatium is a colourless, globular, naked body, with a nucleus which does not appear to be in the resting condition, but can be seen in preparations stained in hæmatoxylon as three black points, which are considered by Ishikawa to be chromosomes.

The carpogonia are formed in areas near the marginal portion of the thallus. In shape the carpogonium is ellipsoid or ovoid. It shows a slight protuberance which is possibly of the nature of a rudimentary trichogyne. It was found that the liberated spermatia attach themselves to the trichogyne, but the critical stage of the passage of the male nucleus through the trichogyne and subsequent fusion with the carpogonial nucleus was not observed.

After fertilisation the zygote divides into eight cells and the process of division follows the same course as spermatium formation. Each cell of the octant subsequently assumes a spherical shape and becomes a carpospore.

Porphyridium. The systematic position of *P. cruentum* has been a much-disputed point, and it has been removed from one
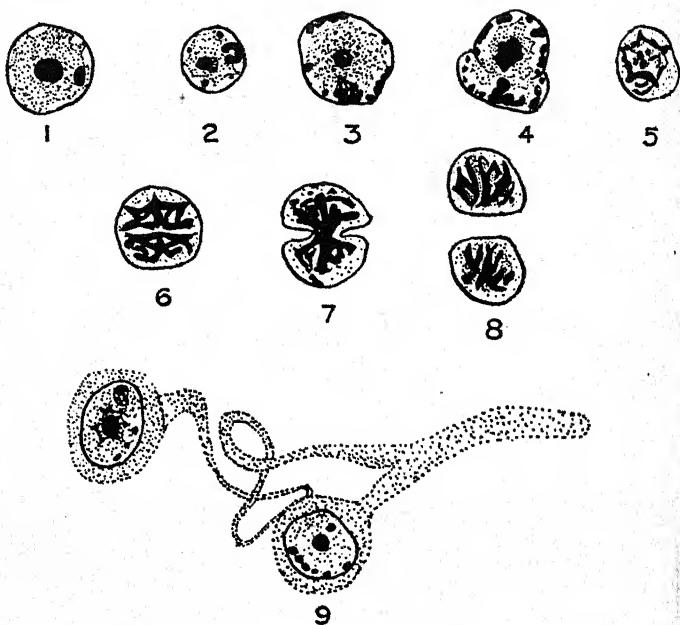
Fig. 48.—*Porphyridium cruentum*. (1) to (8). Stages in the fragmentation of chromatin and changes in pyrenoid, as well as cell division. (9). Cells connected by stalks. (After Lewis and Zirkle, *Amer. J. Bot.*)

group of Algæ to another and been placed at various times in the Chlorophyceæ, Cyanophyceæ and Bangiales. The description of the plant has also been variously given by different authors.

Lewis and Zirkle have re-investigated the matter and are of the opinion that *P. cruentum* should be included in the Bangiales.

P. cruentum is a unicellular organism with a secretion of jelly or mucilage round each cell. At the time of division the two

daughter cells are at first enclosed in the common gelatinous sheath, which follows the constriction of the cells quite intimately and lengthens as the cells draw apart and the portion between the two daughter cells becomes drawn out into a long strand or stalk (Fig. 48, 9). Each cell contains a stellate, dark-red chromatophore when in the resting condition, but in actively dividing cells the chromatophore becomes more amœboid in shape. The presence of a single centrally placed globule has frequently been noted by different authors in *P. cruentum.* This body is slightly smaller than the pyrenoid, is readily visible in living cells and has been observed to disrupt as the water-content of the cell increases, the fragments arranging themselves about the chromatophore.

The chromatin content of the cell consists of a single excentrically placed granule surrounded by a clear space (Fig. 48, 1–8). As the cell prepares to divide, this granule enlarges and begins to fragment and at the same time assumes various shapes (Fig. 48). The U-shape is perhaps the most common. As a result of fragmentation, the chromatin becomes distributed in the form of small, elongated granules about the periphery of the chromatophore (Fig. 48). These granules then fuse end-to-end and a tangled spireme results (Fig. 48). This spireme now breaks into a number of pieces of varying lengths and these segments frequently withdraw into two distinct masses before the cell has commenced to constrict. More often, however, the chromatin becomes constricted into two with simultaneous constrictions of the cell (Fig. 48). Two granules of chromatin occur with great regularity at the poles of the dividing cell and the maximum distance from the plane of constriction (Fig. 48). During the whole process of division no trace of spindle fibres is to be seen.

The question whether this body is a nucleus or nucleolus is discussed by Lewis and Zirkle, who point out that the answer depends on the relative flexibility with which these terms are used. The whole process may be likened to a crudely mitotic division, perhaps of a primitive and exceptional kind.

The bearing of the method of nuclear division upon the systematic position of *P. cruentum* must remain uncertain till more is known of the nuclear history of the Bangiales. It will be

remembered that Ishikawa's account of nuclear division in *Porphyra tenera* showed that the process was not normal mitosis.

In the resting stage the cell of *P. cruentum* is unlike anything to be found among the Cyanophyceæ, though in the later stages of division there is some resemblance to this group. On the other hand, the whole process of division is too primitive for the Chlorophyceæ. In its other characters it resembles the Bangiales, and perhaps is at present best retained in this order.

Nemalionales

In the higher Florideæ there is a most efficient alternation of generations, with three kinds of plants, male, female and tetrasporic, and the seat of chromosome reduction is in the formation of the tetraspores. Although morphologically similar, the tetrasporic plants carry the diploid number of chromosomes, while the male and female plants are haploid. In some simple forms there is no alternation of generations, and all grades between are to be found till a definite and regular alternation is reached in the higher forms.

In 1915 Svedelius described the simplest case of all for the species *Scinaia*. No tetraspores are to be found in this form, and carpospores and spermatia are produced on the same plant. Both carpospores and spermatia are cut off from mother-cells at the surface of the thallus, and there was evidence that successive crops were formed from the same mother-cell. Chromosome counts showed that 10 chromosomes were to be found in both spermatia and carpospores, and the similarity in their method of formation suggested them to be homologous structures. The carpogonial branch was three-celled and the terminal cell became the carpogonium. The nucleus divided to give an egg nucleus and trichogyne nucleus. The second cell of the carpogonial branch developed four auxiliary cells, while the cystocarp membrane was formed from the third or basal cell. The egg nucleus was found to contain 10 chromosomes, and after fertilisation the chromosome number in the nucleus of the zygote was 20. Following upon fertilisation, this diploid nucleus passed into one of the auxiliary cells, which in the meantime had become more or less fused together. The reduction division was found to occur at the

first division of the zygote nucleus, and a second division gave rise to a tetrad of four nuclei. Three of these nuclei took no further part in the life-history. The gonimoblast filaments that were subsequently formed showed nuclei each with 10 chromosomes, and this number was passed to the carpospores, which were ascertained to be formed successively in rows, while some of the gonimoblast filaments remained sterile and developed long hairs which rather resembled paraphyses. It will be seen here that the cystocarp is not fundamentally sporophytic in nature.

In 1904 Wolfe, in his description of the life-history of *Nemalion multifidum*, described the reduction division as taking place with the formation of the carpospore, *i.e.*, that the cystocarp was sporophytic. A re-investigation of the life-history by Cleland has shown that this statement is incorrect and that, as in *Scinaia*, the reduction in chromosome number takes place at the first division of the zygote.

The procarp in *N. multifidum* arises laterally from a vegetative tuft. It is easily recognised, even in the one-celled condition, by its form, being much broader and shorter than the vegetative branches. As a general rule, the mature branch consists of four cells, but five- or three-celled branches are not uncommon, and even the two-celled condition is to be found on occasion. After the procarp has attained its full size the terminal cell gives rise at its tips to a swelling, which elongates and develops into a trichogyne. Unlike *Scinaia*, it is rare to find a trichogyne nucleus. At the base of the carpogonium is found the egg nucleus and a chromatophore lies above it.

At the time of fertilisation, both the spermatial and the carpogonial nucleus are in the resting condition, but soon after the fusion of the spermatium with the trichogyne, the spermatial nucleus divides and two male nuclei may on occasion enter the trichogyne, but only one succeeds in entering the carpogonium. As the male and female nuclei approach one another in the carpogonium, they can be seen to be of unequal size, the female being one and a half times as large as the male. The actual process of fusion is a prolonged and long-drawn-out affair.

After fusion has taken place, the zygote nucleus increases in size

and then divides, and eight bivalents are to be seen, *i.e.*, the first division of the zygote is a reducing division. The two daughter nuclei lie above one another in the cell, and the chromatophore also divides at the same time and a cell wall is then laid down across the carpogonium, cutting it into an upper sporogenous cell and a lower hypogenous cell. The lower cell undergoes no further divisions, whereas the nucleus in the upper sporogenous cell divides, and nuclear division is followed by the laying down of a vertical wall cutting off a lateral segment. This process is repeated several times, with the result that a number of cells are cut off laterally around the central sporogenous cell. A gonimoblastic filament is developed from each of these lateral cells, and when the mature stage is reached each filament consists of a series of three or four cells, the terminal one of which develops into a carpospore.

When the carpospore is ripe and ready to be shed, the wall ruptures at the distal end and the protoplast escapes, leaving an empty wall attached to the filament. The growth of the filament does not stop with the shedding of the carpospore, but a new cell is budded off within the empty shell and this process is repeated.

Svedelius (1915) divided the Florideæ into two groups, Haplobiontæ, in which there were no tetraspores and no change in the chromosome number of the cystocarp, and the Diplobiontæ, which carry the diploid number of chromosomes through the gonimoblasts of the cystocarps, through the carpospore and through a tetrasporic generation. One striking feature about the tetrasporic plant is that it is morphologically undifferentiated from the carposporic one, *i.e.*, the sexual plant, although in *Galaxaura* there are slight differences.

In the undoubtedly primitive form, *Scinaia*, as well as in *Nemalion multifidum*, tetraspores are never formed, the reduction division occurs immediately after nuclear fusion in the carpogonium, and the cystocarp is haploid. In the Diplobiontæ, on the other hand, the reduction division has been dropped at this stage, the cystocarp itself is diploid and the diploid carpospore germinates to give a diploid plant which forms no sexual organs, and the reduction division is brought about in the formation of

the tetraspores which are produced on these non-sexual plants. Germination of the tetraspores leads to the production of male and female plants. Through postponement of the time and place of the reduction division, a new generation has been brought into existence, or in Svedelius's own words (1927) : " By delay in the time of the reduction division in the Florideæ, a new generation may arise at a stroke." The cause is unknown, and the suggestion is put forward by Svedelius that it may be due to hormones. It might also be asked why hormones should be singled out for this distinction, as they already carry at the present time a sufficient burden of explanations of diverse physiological phenomena.

Nevertheless, it appears clear that in the Florideæ the sporophytic generation has not arisen by progressive sterilisation, as Bower's and Čelakovský's antithetical theory demands, but at once. This fact, however, does not completely exclude the occurrence of interpolated organs. Svedelius considers that the Floridean cystocarp has arisen in this way, *i.e.*, by interpolation, through the later formation of sporogenous filaments from the zygote by a process of sterilisation, whereas the zygote in the more primitive Florideæ (as still happens in the Bangiaceæ) was merged in spore formation.

The question arises here, Why should the Floridean body which arose so suddenly in the evolution of this group possess no sexual organs ? It has been suggested that the diploid chromosome number may in some way hinder their formation.

Abnormalities in the Production of Reproductive Organs in the Florideæ

Various investigators from time to time have noticed and recorded the appearance of reproductive organs typical for both haploid and diploid generations, on one and the same plant in certain members of the Florideæ.

In 1906 Yamanouchi, in his classical investigation of the cytology of *Polysiphonia violacea*, recorded the presence of cells on the sexual plants which he considered to be of the same nature as tetraspores, although they apparently never matured. But cases have also been found in which mature asexual spores were formed

on sexual plants—for example, *Callithamnion Bialeyi* by Davis, and *C. tetragonium* by Harvey-Gibson and Knight.

A detailed investigation of this problem has been made by Mathias on *Callithamnion brachiatum*. *C. brachiatum* belongs to the Diplobiontæ of Svedelius, *i.e.*, ordinarily the diploid plants form tetraspores, while the sexual plants, male and female, carry the haploid number of chromosomes.

It was found that normally the reduction division took place at tetraspore formation, and the ordinary life-cycle of *C. brachiatum* is quite typical of the higher Florideæ, the haploid tetraspores
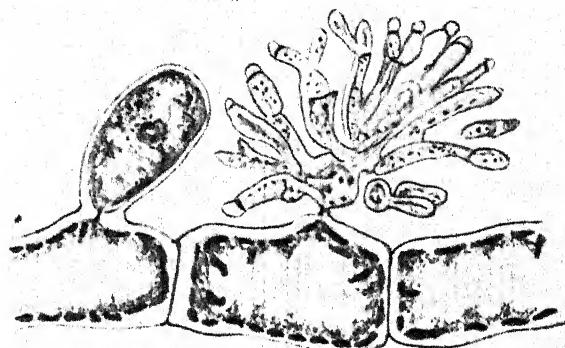
FIG. 49.—*Callithamnion tetragonium*. Portion of a filament bearing a group of spermatia and a young tetrasporangium on adjacent cells. (After Mathias, *Publ. Hart. Bot. Lab.*)

developing to give haploid sexual plants, and a diploid plant is the result of fertilisation.

But fully mature spermatangia and tetrasporangia were frequently found to be present on the same plant of *C. brachiatum* and were borne side by side (Fig. 49). Unfortunately the cytology of their development was not followed. Similarly, cystocarps and tetrasporangia were also found on one and the same plant and, in the case of the tetrasporangia, the cytology of their development proved to be entirely normal. But the nuclei of the carpospores were found to be haploid, whereas the chromosomes in the supporting filament are diploid. It is probable,

therefore, that a reduction division occurs during the formation of the cystocarp. The existence of a carpogonial branch was not observed prior to the formation of such cystocarps, but as the carpospores are in twin groups, as in the typically sexual plants of *Callithamnion*, the probabilities are that a carpogonial branch antedated their formation. If the origin of such cystocarps should prove to be apogamous, a reduction division has yet to be proved either in the carpogonial branch or in the auxiliary cells. If, on the other hand, such carpospores are the result of fertilisation prior to their formation, since they are carried on a diploid plant, a double reduction division must take place, such as that described for certain of the Ascomycetes by Fraser and others (see Chapter IV). Whatever be the result of further investigations, the observation of haploid carpospores on a diploid thallus is unique for *Callithamnion*.

The Life-history and Taxonomic Position of Certain Selected Florideæ

Sturch has described the life-histories and discussed the possible taxonomic positions of three parasitic Florideæ : *Harveyella mirabilis*, *Harveyella (Holmsella) pachyderma*, and *Choreocolax Polysiphoniæ*.

H. mirabilis is a parasite on *Rhodomela subfusa*, while *H. pachyderma* is a parasite on *Gracilaria confervoides*. These parasites form small, white cushions, somewhat hemispherical or slightly flattened in shape, and are attached to their host by a short, thick stalk. They lack chlorophyll and absorb their food from the cells of the host plant. Branched filaments grow down among the cells of the host and act as haustorial organs, while externally they give rise to the white cushions that have been described above.

H. pachyderma is abundant from November to March on *Gracilaria confervoides*, and plants bearing antheridia, procarps and cystocarps are produced from November to February. The tetrasporic plants make their appearance in January and are produced till March.

Although in their external morphology these two parasites are very similar, they differ in important features in their reproductive

processes, and it is on this account that Sturch has removed *H. pachyderma* from the genus *Harveyella* and placed it in a new genus, *Holmsella*.

The procarp in *H. pachyderma* is cut off from a cell of the peripheral layer. The cell below the procarp continues to function as a peripheral cell, while the procarp grows downward at the base and cuts off a small cell. No further cells make their appearance and the carpogonial branch remains two-celled. Four-celled and three-celled carpogonial branches have been observed by Sturch, and it is therefore possible that before the parasitic method of life was assumed, *H. pachyderma* normally possessed a four-celled carpogonial branch. After fertilisation the zygote sends out a short primary ooblastema filament, which is occasionally two-celled. At about the same time the auxiliary mother-cell, which is one of the lower cells of a near peripheral chain, divides by a transverse wall into a lower basal and upper auxiliary cell. Occasionally this auxiliary cell pushes out a small projection towards the approaching ooblastema and fusion takes place, and the first stages in the development of the fusion cell are very similar to that found in the Ceramiales. The fusion cell divides transversely into a lower foot cell and upper central cell, the latter containing a nucleus descended from the zygote. This nucleus now divides rapidly and secondary ooblastema are formed. At the same time, cells at the base of the periphery commence to elongate, and in all cases this process begins directly after the fusion of the primary ooblastema with the auxiliary cell, the first cells to lengthen being connected primarily, or secondarily, with the basal cell. A few cells behind the advancing distal end, the cells of the horizontal ooblastema begin to develop gonimoblastic clusters of carposporangia branching in all directions.

Harveyella mirabilis differs from *Holmsella pachyderma* in the carpogonial branch, which, without exception, is four-celled. The peripheral cell supporting the carpogonial branch acts as an auxiliary cell and there is no further division into basal and auxiliary cells as in *H. pachyderma*. This auxiliary cell cuts off two further small cells, which develop into short sterile branches, one of four cells and the other of two cells. After fertilisation,

fusion takes place by means of a short ooblastema and a central cell is cut off, while the lower part—the foot cell—and the cells of the sterile branches widen their connections, and eventually become fused into a large cœnocytic cell.

Since these two parasites differ in all the details of nourishment of ooblastema, essential mechanism of ooblastema fusion, and the secondary fusions so common in *H. pachyderma* being absent in *H. mirabilis*, they are sufficiently distinct to be placed in different genera.

The life-history of *Choreocolax Polysiphoniæ*, which is completely parasitic on *Polysiphonia fastigiata*, has also been fully investigated by Sturch (1926). *Ch. Polysiphoniæ* is a full-cycle member of the Florideæ, with antheridia, procarps, and tetrasporangia, on separate plants.

In external appearance it is a small cushion-like plant, and its relation with its host, *P. fastigiata*, in this instance, is of interest. *Ch. Polysiphoniæ* is completely parasitic on *P. fastigiata*, and the latter is itself a partial parasite on the Fucoid, *Ascophyllum nodosum*.

As a reproductive machine *Ch. Polysiphoniæ* is a highly efficient organism. The power of photosynthesis has been lost and the free soma has dwindled to such an extent that it practically occupies a space but little larger than that taken up by the cystocarps, and in these circumstances it is able to produce a large number of spores with the minimum amount of somatic growth.

The procarp is initiated from a distal cell of a peripheral ramulus and develops in the ordinary way. The young procarpium is noticeably larger than the peripheral units surrounding it. At first the development of the trichogyne keeps pace with the growth in length of the peripheral ramuli, but soon passes beyond them, growing through the gelatinous outer membrane until it reaches the external medium. The carpogonium sends down a basal prolongation and three small cells are segmented off, and the carpogonial branch invariably consists of three cells and a trichogyne. The procarps are fairly well distributed throughout the peripheral layer, and as they become old and buried in the soma, new ones arise.

8—2
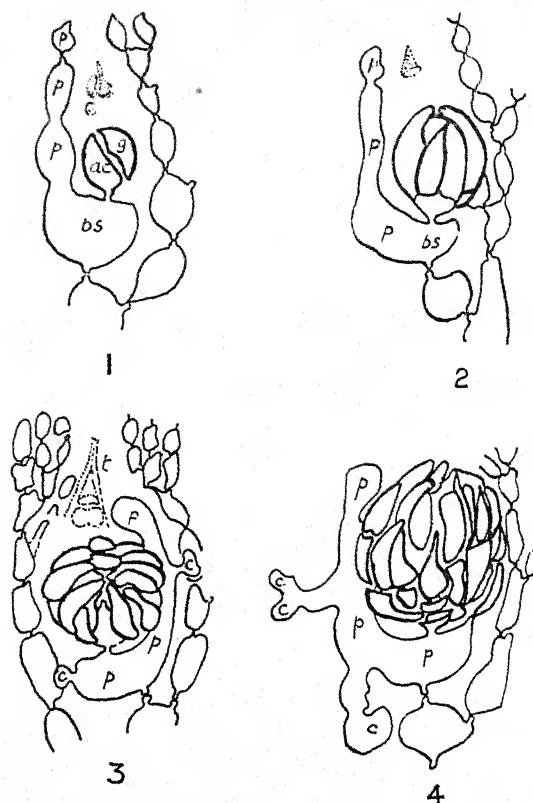
FIG. 50.—*Choreocolax Polysiphoniæ.* (1). Development of auxiliary cell. *g*, first gonimoblast cell; *ac*, auxiliary cell; *bs*, basal cell; *p. p. p.*, cœnocyte. The outlines of the cells of the cœnocyte are shown thickened. (2). Very young stage of cœnocyte. *bs.*, basal cell; *p. p. p.*, cœnocyte. (3). Complete young carpo-sporophyte. *p. p. p., c. c.*, further development of cœnocyte; *t*, relic of procarps. (4). Later stage in development of carpo-sporophyte and of cœnocyte (*p. p. p., c. c. c.*). (After Sturch, *Anns. Bot.*)

In *Choreocolax* the peripheral cell supporting the procarp is the auxiliary mother-cell. This divides to give an auxiliary cell and a basal cell, but only after the trichogyne has reached the exterior, and never before. The basal cell in turn forms a short

ramulus of apparently ordinary peripheral cells growing towards the surface. When fertilisation has taken place, the zygotic nucleus is transferred to the auxiliary cell by a very short primary ooblastema, the base of the carpogonium being at this time in close proximity to the auxiliary cell. Development takes place rapidly in some stages, with apparently one period of rest before the cystocarp reaches maturity (Fig. 50).

The auxiliary cell cuts off a shoulder by means of an oblique curved wall, and thereby forms the first cell of the carposporophyte. At the same time the small ramulus attached to the basal cell enlarges its cell connections and is termed by Sturch the cœnocyte. Up to this stage the secondary connections so common between carposporophyte and somatic cells are absent.

The carposporophyte continues to grow and forms a more or less flattened, spherical mass of closely packed cells. When mature it consists of a reticulum of interlacing filaments composed of elongated, rather irregular cells, forming a hollow, spherical zone, on the inner surface of which the gonimoblasts develop as short tufts, bearing the carposporangia.

Sturch considers that *Harveyella mirabilis, Holmsella pachyderma* and *Choreocolax Polysiphoniæ* are to be regarded as " strays " of three different groups which have managed to hold their own owing to their success as parasites. They probably began as Florideæ with a fully elaborated reproductive cycle, and, in spite possibly of poor and inferior somatic equipment have saved themselves in the struggle for substratum by the adoption of a parasitic mode of life. It may not therefore be surprising if these parasites should not be found to fit in very well with the present scheme of Floridean classification.

The present system of classification among the Florideæ is based on auxiliary cell attainment in a large number of genera. These three forms do not fit well into the Nemaliones, in which the fertilised carpogonium itself gives rise directly to the gonimoblast system of the carposporophyte. In the higher Florideæ, Gigartiniales, Rhodymeniales and Ceramiales, true auxiliary cells serve as starting-points for gonimoblast formation.

These three parasites all possess definite auxiliary cells from

which gonimoblast formation is initiated. *H. mirabilis*, with its auxiliary cell definitely segmented after fertilisation, is definitely one of the Ceramiales. The same applies to *Holmsella* and *Choreocolax*. Nevertheless, all three possess carposporophytes which resemble to a great extent those of certain members of the Nemaliones, but however much they may resemble the carposporophytes of the latter, the presence of auxiliary cells makes their inclusion impossible in this group. Sturch proposes that a new family, the Choreocolaceæ, should be made provisionally to hold these three somewhat aberrant forms, and he defines the family as follows : Holoparasitic Florideæ, without chlorophyll. Somatic organisation is of the cable-strand type, but very much reduced.

Another difficult genus to place, and one which can be conveniently considered here, is *Gracilaria*. The life-history of three species, *G. multipartita*, *G. confervoides* and *G. compressa*, has been investigated by Phillips (1925).

Ordinarily in the Florideæ, a procarp consists of a specialised carpogonial branch, ending in a carpogonium carrying a trichogyne and with one or more auxiliary cells. Phillips was unable to discover any definite hair-like structure corresponding to a trichogyne in these species, but, lying among the superficial cells some large cells could be observed with no well-defined chromaphore and devoid of colour. These cells do not protrude appreciably from the surface of the thallus, but extend downward as to be among the intermediate cells with which they show the connections. In tangential section as well as in vertical raised papilla-like areas can be seen, somewhat resemprocarps of *Porphyra* among the Bangiales. These larg cells with their raised papilla-like areas are the carpogo are differentiated from behind the growing-points of

After fertilisation the carpogonium appears stimulus to all the superficial cells in the neig nearest being affected first, until gradually t gonium is overtopped by the growth of the su cells and the zygote becomes immersed a liferated tissue. It now enters on a ca exercises a remarkable effect on the b

which immediately surround it, pushing these cells back and fusing with a great number of them and absorbing their contents. Pseudopodia are sent out and these cells become gradually incorporated into the substance of the zygote.

No specialised auxiliary cells are present, but absorption and incorporation involves the destruction of many cells and the zygote may be described, after Church, as a "trophocyte." Protusions are now formed upwards and outwards and each divides into groups of cells which become the apical cells of a prolific branch system of new carposporophytes. Ultimately carpospores are formed at the surface of the carposporophyte within a dome-shaped cavity.

Phillips somewhat tentatively suggests that *Gracilaria* should be removed from its present position among the Ceramiales and be placed near the Bangiales.

Another difficult form to place is *Spyridia filamentosa*, whose life-history has also been investigated by Phillips (1924). *S. filamentosa* is a widely spread species, and antheridia, procarps and tetrasporangia occur on different plants, *i.e.*, it belongs to Svedelius' Diplobiontæ. In general external appearance it is somewhat similar to *Polysiphonia*. Little clue, however, is to be nd in the structure of the thallus as to affinities. The method as ortication of axes of unlimited growth is peculiarly its own, on hows some resemblance in the cortication of axes of limited imp to *Ceramium*, but these occur upon structures which ave the pi that genus.

velopment of the cystocarp is critical for purposes of Virus n, and here the procarp arises from adventitious axes ledons, a growth, but which become arrested after fertilisation regions. o has taken place. The procarps arise singly on of, if not a of the main axis and consist of a four-celled time, til h arising from a pericentral cell, but only one son, s, to a cystocarp. A large auxiliary cell is formed C were pr taken place and the carpogonium fuses with about 0° C., a s s pary cells are formed, one primary and three layer of liquid ees gonium fuses with the primary auxiliary more acetone, er ary auxiliary cells actively initiate conju- obtained was dis ns,

gation tubes which reach the primary auxiliary cell and then become the seat of the origin of sporogenous threads. Gonimoblasts grow out from the secondary auxiliary cells and are terminated by chains of carpospores.

If emphasis be laid on the fact that the secondary auxiliary cells send out processes to the primary auxiliary cell and that the reverse process does not take place, it is impossible to find a place for *Spyridia* in the existing schemes of classification, for no such process has been described for other Florideæ.

Structure seems to indicate an affinity with the Ceramiaceæ. Cortication, however, is unlike either *Ceramium* or *Polysiphonia*. Nor does an examination of the intimate structure of the cystocarp reveal similarities to any of the families of the Rhodymeniales. It is true that, like the Rhodymeniales, it has a fixed procarp, but it must also be borne in mind that, unlike them, it possesses secondary auxiliary cells which are the seats of spore formation. Phillips favours the Gigartiniales as showing the nearest affinities to *Spyridia*, and makes the suggestion that it may perhaps find a place near *Wrangelia*. In *Wrangelia* the result of fertilisation of one procarp is communicated to the basal cell of other branches of the same whorl, and ultimately to similar cells of other whorl above and below. It is not inconceivable that *Spyridia* may be specialised condition of what is a more diffused state in *Wrangelia*. On these grounds, he suggests that *Spyridia* be placed in a spcial as to the among the intermediate cells the Wrangeliaceæ if the connections. In tangential section as well as in vertic raised papilla-like areas can be seen, somewhat reser procarps of *Porphyra* among the Bangiales. These lar cells with their raised papilla-like areas are the carpog are differentiated from behind the growing-points of

After fertilisation the carpogonium appears stimulus to all the superficial cells in the neigh*lingar. Bd.*, **63**. nearest being affected first, until gradually th gonium is overtopped by the growth of the su*t. Mag.*, **34**. cells and the zygote becomes immersed at t liferated tissue. It now enters on a co*psaliensis*, **4**; (1927) *Bot.* exercises a remarkable effect on the ba

THE VIRUS DISEASES OF PLANTS

Introduction—Symptoms—Properties of Plant Viruses—Number and Specific Nature of Plant Viruses—Transmission—Carriers— Ætiology—Physiology of Virus Diseases.

Introduction

THE last decade has witnessed extensive investigations into the virus diseases of plants, and the number of Institutes and Research Stations in this country and America engaged upon work on this aspect of plant pathology has increased year by year.

It was in 1892 that Iwanowski gave the first demonstration of filterable viruses, either in plants or animals, when he showed that the causal agency responsible for the mosaic disease of tobacco was able to pass through a Chamberland candle. From that time, as far as the virus diseases of plants are concerned, knowledge only slowly accumulated till within recent years, when the economic importance of these diseases became increasingly apparent, and at the present time the annual volume of published work has reached very large dimensions.

Virus diseases attack Dicotyledons as well as Monocotyledons, and these diseases are prevalent in tropical and temperate regions. Although there is little reason to doubt that the majority of, if not all, virus diseases have been in existence for a very long time till comparatively recent years they were confined over small areas, but increased methods of travel and commerce have were present wide distribution.

about 0° C., a plants are more prone to attack by virus diseases layer of liquid ees ; the reason is at present unknown. They occur more acetone, er cultivation, and also in plants growing under obtained was dis ons, and the annual loss in such economic crops as

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the tobacco, potato, cucumber, sugar-cane and sugar-beet, on account of infection by these diseases, amounts to many millions of pounds.
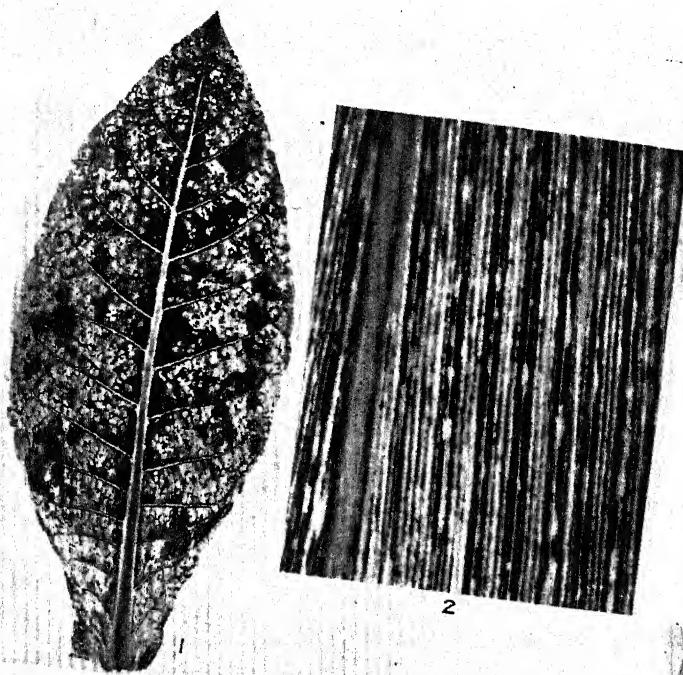
Fig. 51.—Examples of virus diseases of Plants. (1). Leaf of tobacco (*Solanum tabacum*) infected with mosaic disease. (2). Streak disease of maize (*Zea Mays*). Portion of streaked maize leaf showing chlorotic stripes following the leaf veins and frequently fusing laterally. ((1). After McMuntrey, *J. Agric. Res.* (2). After Storey, *Anns. Appl. Biol.*)

Symptoms

The effect of these diseases varies greatly. As a rule there is stunting of the plants, distortion of the foliage; nearly every instance some chlorosis, and in the case of leafy crops there is a reduction in yield. The most constant symptom is chlorosis. There is a mottling of the leaves in the case of

Allard (1915) showed that dilution up to 1/1000, and even 1/10,000, did not lead to loss in the power of infection of the diseased juice of tobacco mosaic. This virus is very resistant. Nitric and hydrochloric acid had little effect, except in concentrations of 1 gm. in 50 to 100 c.c. of virus solution. Similarly, phosphoric, citric and acetic acid had no effect except in concentration of 1 gm. in 200 c.c. of virus solution. The virus was found to be more sensitive to alkalis, such as sodium hydroxide or sodium carbonate, while salts, such as manganese sulphate, sodium chloride, silver nitrate and mercuric chloride, had little effect upon infectivity. Copper sulphate, however, in a concentration of 1 gm. in 100 c.c. of water was toxic. Antiseptics, with the exception of 4 per cent. solution of formaldehyde which destroyed the virus, had little effect.

Tobacco mosaic is also very resistant to high temperatures, and in certain cases will still retain its power of infection after being kept for as long as two days at 80° C. Higher temperatures than this (85° to 90° C.) will, however, generally inhibit its action in ten minutes. Sunlight has no effect upon it, and it was found to resist the action of X-rays for half an hour and the rays of a mercury-vapour lamp for an hour.

It passes with ease through the finer Berkefeld and Chamberlain porcelain candles, and Mulvania showed that it could even pass through collodian membranes of a definite grade of porosity, but that this latter feature depended upon the acidity of the sap.

Tobacco mosaic will also withstand drying over sulphuric acid, and the sap has been found by Dickson (1925) to be still infective after simple storage for five years, while Valleau and Johnson have found that dried and cured tobacco leaf is still infective after thirty-one years.

The effect of acetone on tobacco mosaic has been investigated by Vinson, who found that when two volumes of acetone at — 8° C. were added to one volume of the expressed sap held at about 0° C., a flocculent precipitate was thrown down. The upper layer of liquid was decanted off, and the precipitate washed with more acetone, and finally with ether. The precipitate thus obtained was discovered to be readily soluble in water, and seed-

lings infected with this solution showed it to be highly infectious. The first supernatant liquid which had been decanted off proved to be non-infective. Absolute alcohol could also be used under the conditions mentioned above in place of the acetone, and infection was still produced, so that neither of these reagents have any effect upon the virus at low temperatures, and Walker has shown that alcohol up to 90 per cent. does not destroy the virus even after being allowed to be in contact for one hour.

According to Vinson and Petre the virus of tobacco mosaic can be freed from 90 per cent. of non-active solids of the juice with no loss in infective power. It was found that an aqueous extract of safranin (1 per cent. in the proportion of 10 to 50 c.c. of the plant extract) precipitates the virus of tobacco mosaic, and this precipitate contains practically the whole of the virus. The virus is inactivated by this treatment, but is released in an active state when the safranin is removed with amyl alcohol. Ammonium sulphate also throws the virus out of solution, while lead acetate and barium acetate in low concentration throw down the majority of colouring matter and proteins without injuring or removing the virus. After clearing with lead acetate and barium acetate, the juice from diseased plants was concentrated *in vacuo* below 50° C. without injury to the virus. When concentrated *in vacuo* to about 0·4 of the original volume and brought to 0° C., two volumes of acetone were added at − 15° C. This precipitate only contained 10 per cent. of the solids of the original juice and apparently the whole of the virus. The fact that, with the proper concentration of safranin, acetone or ethyl alcohol, the precipitation is almost complete, and in each case the precipitate contains practically the whole of the activity of the juice, together with the fact that the virus is apparently held in an inactive condition in the safranin precipitate and is released when the safranin is removed, inclines Vinson and Petre to the view that the virus they investigated reacted as a chemical substance.

Other plant viruses have been found to possess different properties to tobacco mosaic. The viruses of aucuba mosaic of tomato and ground-cherry are very similar in their properties to tobacco mosaic, and appear to be highly resistant to external agents, but

potato mosaic is very much less resistant. In one particular strain of potato mosaic it was found by Henderson Smith (1928) that the virus was inactivated in ten minutes by a temperature of 80° C. and by 90 per cent. alcohol in one hour. The virus of cucumber mosaic also differs from tobacco mosaic. It is filterable through Berkefeld, but not through Chamberlain filters, and is quite unable to withstand drying, and loses its power of infection after standing for seven days. A temperature of 70° C. inactivates its power of infection, while as weak a strength of alcohol as 45 per cent. also destroys it.

According to Walker the properties of a virus are largely determined by the properties and characteristics of the host sap. He found, for example, that when cucumber mosaic was transmitted to *Physalis* (ground-cherry) and its properties studied in the new host, it had lost its somewhat unstable character, and conversely *Physalis* mosaic transferred to cucumber acquired the unstable properties of cucumber mosaic.

Both McKinney and Holmes have emphasised the importance and desirability of a standard procedure for quantitative studies in the investigation of virus extracts. Thus, the usual method of testing the effect of dilution on a virus extract is to inoculate from ten to fifty plants in one of the accepted ways of mechanically transmitting the disease, and then to judge from the resulting number of infections which of two sources contained the larger proportion of virus in a given volume. This method is perfectly suitable when it is simply a question of deciding between strong and weak samples of virus, but when it is a question of deciding between samples which only differ slightly in strength, these can only be graded and differentiated by inoculating larger numbers of plants, and this is not always convenient. Holmes has recently laid down rules for determining more accurately on a quantitative basis the properties of a virus, and the original should be consulted for the full details.

The Number and Specific Nature of Plant Viruses

A satisfactory method of classifying and identifying plant virus diseases has yet to be found, and would be a very great convenience.

At the present time the investigator is almost entirely dependent on symptomatology, and this among mosaics, especially potato mosaics, is highly confusing.

Certain hosts are susceptible to several distinct diseases, *e.g.*, sugar-beet, which can be attacked by curly-top of beets and beet mosaic, and the potato, which can have leaf-roll and mosaic. Each of these diseases on transfer comes true to type ; thus beet mosaic does not give rise to curly-top, and leaf-roll in the potato does not give mosaic or *vice versâ*. In such cases it can hardly be doubted that these diseases are in themselves distinct, and it seems certain that there are several different plant viruses.

The chief difficulty lies in the distinction of these diseases *inter se*. Thus, how many mosaics of tobacco are there ? Johnson recognises eight different mosaics in tobacco. Are these eight different mosaics really eight different diseases, due to eight different viruses, or are they merely modifications of a single virus ? The question is unanswerable in the present stage of our knowledge.

The virus diseases of the potato have been the cause of endless confusion with regard to classification and number. In 1923 Schultz and Folsom described five, possibly seven, different virus diseases which occur in the potato. Confusion here, however, has now reached an acute stage, because it has been shown that certain of these diseases are composite in character, and the letters of the alphabet have been freely employed to designate the various portions of these composite diseases. Such a procedure in itself is not to be deprecated, since it is difficult to see what other system can be employed in the circumstances, but there is no excuse to rename well-established and well-recognised diseases on quite flippant and unnecessary grounds because an investigator thinks it probable that in the near future some other disease may be found similar to it. A case in point is the crinkle disease of potato. This is a well-known and familiar disease to all workers on virus diseases of the potato, and its symptoms have been clearly described by Murphy and Quanjer. It is now called crinkle " A " by Salaman, as he has found an analogous disease which differs somewhat from it in a latent condition in healthy King Edward

plants. Crinkle " A " is Murphy's crinkle or merely crinkle and nothing else, and to tag on the letter " A " will not help one iota in solving its nature.

Although symptom expression will always remain an important diagnostic feature with virus diseases, symptoms in themselves cannot be regarded as a reliable index of the virus concerned in the majority of cases where diagnosis is required, owing to the similarity and overlapping of symptoms produced by different viruses, their extensive modification by environmental factors and other circumstances. Nor is it likely that any one single diagnostic feature will meet all the requirements of the case. If, however, as Johnson (1929) has suggested, several unrelated diagnostic features be taken into consideration, a process of elimination or " key " may be obtained which will reduce the possibility of error to a minimum for purposes of classification.

The particular features used by Johnson are thermal death-point, the longevity *in vitro*, the effect of dilution and the influence of certain chemicals. Several other characteristics that could also be used are : method of transmission required, length of incubation period, comparative readiness of transmission by a single method, *e.g.*, grafting, insects, leaf mutilation, host range in different species or varieties, relation of source of inoculum to infection, influence of environmental conditions on symptom expressed, variation in cytological and histological details, and filtrability.

Transmission

Certain plant viruses are transmitted with extreme ease, *e.g.*, tobacco and cucumber mosaic, and in both these diseases the causative agents are filter-passers. It will be remembered that it was shown by Allard that juice from tobacco plants infected with mosaic when diluted to 1 in 10,000 parts of water was still able to cause infection. Tobacco mosaic is spread with great readiness, even the rubbing of the fingers on a diseased plant and then rubbing a healthy individual is quite sufficient to cause infection. The virus here is also highly resistant, and it has been shown that juice allowed to ferment and kept at room temperatures for as

long a period as twelve months was still infective, while in dried tobacco leaves the causal agent will retain its power of infection for several years. Nevertheless, in spite of its highly infectious nature, the virus is only capable of entering the plant through wounds. The breaking off of a few hairs or trichomes, or the prick of a needle, is all that is necessary to cause the entry of the infecting agency.

Grafting of diseased scions on to healthy plants is an invariable method of transmission and is much used in certain virus investigations. In fact, grafting is the only known method at the present time of transmitting certain of these diseases, *e.g.*, peach rosette, *Abutilon* mosaic, peach yellows and spike disease of sandal.

Transmission through the seed is not common, but infection through seed has been definitely proved in the case of lettuce mosaic by Jagger, and the mosaic disease which attacks a number of leguminous crops has been shown to be seed-carried by Dickson (1922). There is also a good deal of evidence that potato mosaic can be transmitted through the seed. The best-known case of seed-transmission of a virus disease is probably that of cucumber mosaic, which has been fully investigated by Doolittle and his co-workers. Cucumber mosaic has a very wide host range and can be transmitted to a number of other dicotyledonous plants, such as tobacco, potato, milkweed (*Asclepias syriaca*), pokeweed (*Phytolacca decandra*) and the wild ground-cherry (*Physalis*, sp.). The disease is only seed-transmitted in the case of the wild cucumber, *Micrampelis* (*Echinocystis*) *lobata*, but is transmitted to the cultivated variety by the melon aphis, *Aphis gossypii*. The striped cucumber beetle *Diabrotica vittata* also transmits the mosaic is not to the field.

Undoubtedly the chief agents for the transmission of virus diseases in the field are insects, and the study of the relationship between virus disease and insect vector has now reached large dimensions. Kenneth Smith (1931a) has recently published a summary of the subject, and the reader is referred to this résumé for a very complete list of virus diseases and their insect vectors. Only three or four special cases will be considered here.

The virus disease of the potato known as leaf-roll has been

much investigated from the point of view of transmission by insects. Leaf-roll, up to the present time, has not been transmitted by inoculation of expressed sap, but it can be transmitted to healthy potato plants by grafting. It was shown in 1920 by Oortwijn Botjes that when a combination of aphids, *Myzus persicœ*, Sulz, and *Aphis rumicis*, L, were transferred under controlled conditions from diseased to healthy plants, the latter in the course of time developed the disease. This work was later confirmed by Schultz and Folsom, who used the combination *Myzus persicœ* and *Macrosiphon solanifolii*, and regularly secured infection under controlled conditions. Murphy was also able to secure infection with *Myzus persicœ* when the aphid was placed on the sprouts of healthy tubers. Murphy and M'Kay later claimed that a number of other insects were able to bring about infection, *e.g.*, *Myzus pseudosolani*, *Macrosiphon solanifolii*, and the capsid, *Calocoris bipunctatus*.

The whole question of insect transmission in potato leaf-roll has now been very fully investigated by Kenneth Smith (1929, 1931b), who has shown that the transmitting agency, *par excellence*, is the aphid *Myzus persicœ*. The following insects were used: HEMIPTERA, HETEROPTERA, *Capsidœ*, *Calocoris bipunctatus* and *Lygus pabulinus*. HOMOPTERA, *Jassidœ*, *Eupteryx auratus*, *Chlorita viridula*. *Aphididœ*, *Macrosiphon gei* and *Myzus persicœ*. COLEOPTERA, *Psylliodes affinis* (Potato flea beetle).

In all cases, with the exception of *Myzus persicœ*, negative results were obtained. *M. persicœ*, on the other hand, was an efficient carrier, and a large percentage of infections were obtained. It has now been shown by Whitehead that *Myzus circumflexus* is also an efficient transmitter of leaf-roll, and he claims that it is almost as efficient as *M. persicœ*, but there is a curious lag in the development of the symptoms, so that plants infected with leaf-roll by means of *M. circumflexus* will still be in the " primary " stage when those infected with *M. persicœ* show rolling in both the lower as well as the upper leaves.

The question of leaf-roll infection by *M. persicœ* has been carried a stage further by Kenneth Smith. He found, for example, that the action of this aphid is selective. In potato plants affected

with combinations of viruses, such as leaf-roll and streak, or leaf-roll and mosaic, only leaf-roll was transmitted by *M. persicæ*. In the case of the combination, leaf-roll and mosaic, the insect picked up both diseases, but only the leaf-roll was transmitted to potato. That mosaic was also taken up, though not transmitted to the potato, was shown by the fact that when the aphids were transferred to tobacco, the latter developed a mottle.

Since both *M. persicæ* and *M. circumflexus* are able to transmit leaf-roll, it is clear that there is no absolute affinity between *M. persicæ* and leaf-roll, but that this aphid appears to be specially suited to its dissemination cannot be denied, and it is very difficult at the present time to suggest any good reason why one species of aphid should be more suitable for transmission than another. Methods of feeding apparently have little to do with the matter, because *M. persicæ* and *M. circumflexus*, which both transmit, and *Aphis rhamni* and *Macrosiphon gei*, which do not, are all phlœm feeders. It is possible that if the saliva of the insect be very toxic to the plant, then the insect is less likely to act as an efficient transmitter of virus disease, owing to the local disorganisation of the plant cells at the point of entrance of the insect's stylets. If the virus, on injection, be surrounded by a mass of disintegrating and dying cells, the chance of successful infection is very considerably lessened. It may well be on this account that capsid bugs play but a small part in virus transmission, as their saliva is exceedingly toxic to plant tissues in certain instances. Both *M. circumflexus* and *M. pseudosolani*, but especially the latter, produce great visible disturbances in the plant tissue when feeding, whereas *M. persicæ* does not produce such a marked effect.

It was found by Kenneth Smith (1931b) that non-infective aphids (*M. persicæ*) can pick up leaf-roll from an infected potato plant after six hours' feeding, and the infective aphids can transmit the disease to healthy potatoes after two hours' feeding. Thus, the whole process whereby a non-infective aphis picks up leaf-roll virus and infects a healthy plant cannot be carried out in less than eight hours, and a minimum period of some fifty-four hours appears to be necessary. The incubation period of the leaf-roll virus in

the plant before the first symptoms made their appearance was, on the average, fourteen days. It is possible that temperature may play some part in determining the length of the incubation period, because the present author in some experiments on transmission of leaf-roll with *M. persicæ* in the early part of the year (March) found that between twenty-eight and thirty days elapsed before the symptoms made their appearance.

It is possible that virus diseases that are highly infectious may be transmitted mechanically by almost any insects that may happen to visit and feed upon diseased plants and then pass on to healthy ones, but, nevertheless, in certain virus diseases there is a very definite specificity between disease and insect vector. An interesting case in point is that of spinach blight. Spinach blight is one of the few virus diseases that is fatal to the host plant. It was shown by McClintock and Smith that the aphid, *Macrosiphon solanifolii*, is apparently specific for the disease. It was discovered that the spinach blight virus is retained by the aphid for some time when colonised on non-susceptible plants, and that the disease is inherited up to the fourth generation by individuals parthenogenetically produced.

Allard some years ago (1914) put forward the claim that *Myzus persicæ* was an efficient transmitter of tobacco mosaic. This statement has been re-investigated by Hoggan, who found that this aphid was apparently unable to transmit the disease. Out of 395 plants tested, only ten developed the disease, as compared with three out of 395 plants in the controls. It was found that the plants that did show disease had become infected by chance. On the other hand, Hoggan ascertained that *M. persicæ* was an efficient carrier of cucumber mosaic to tobacco. Moreover, the aphid showed selective properties. Colonies reared upon tobacco plants, infected with both cucumber and tobacco mosaic, transmitted only cucumber mosaic from the combination. This selective action of *M. persicæ* points to the fact that the relation between aphid and virus may not be purely mechanical. This result is certainly somewhat surprising, because tobacco mosaic is one of the most highly infectious of plant virus diseases, and even slight injury to the epidermis which results in the introduction

of the smallest amount of virus into the tissues is sufficient to cause infection of the plant with tobacco mosaic, and it is possible that there may be something more involved than mere mechanical transfer of viruliferous juice from plant to plant in aphid transmission of virus diseases.

Although, in a number of examples, one particular species of insect is able to transmit a number of different virus diseases, in other cases, however, there appears to be a specific relationship between certain insects and the diseases which they carry. The " curly-top " disease of sugar-beet is transmitted by the beet leafhopper, *Eutettix tenellus*, and by no other beet-infesting insect, but a mosaic disease of sugar-beet is transmitted by *Myzus persicæ*, and *Eutettix tenellus* is quite ineffective as a transmitting agent.

The streak disease of maize, which is widely prevalent in South Africa, has been investigated by Storey (1925) in relation to spread by insects. The insect concerned here was found to be the jassid, *Balclutha mbila*, Naude. *Balclutha mbila* proved to be a particularly efficient distributor of streak. For example, in one experiment, single individuals were allowed to feed first on diseased plants and then transferred to the leaves of healthy individuals, and the disease resulted in forty-six out of forty-eight plants. Once a jassid had fed upon streak maize plants it carried the disease for a long period. In one special instance a jassid lived for five months and carried the disease to eight separate plants. It was also ascertained that in spite of periods of starvation and of feeding on apparently immune plants, there was no loss of the power of infection. The power of infection was not passed on to the progeny, and the eggs laid by infective leafhoppers gave rise to uninfective offspring, but infective leafhoppers still retained their power of causing disease during change of skin.

It was discovered that the virus needed a period of incubation within the leafhopper. The actual period of incubation was variable and dependent on temperature; the higher the temperature the shorter the period of incubation, and at 30° C. the period was between six and twelve hours.

The rosette disease of pea-nuts, which is also found in South

Africa, has been shown by Storey and Bottomley to be transmitted by the aphid, *Aphis leguminosæ*, and not by leafhoppers. The disease produces striking modifications in the plant, and the diseased individual may become little more than a close tuft of small curled leaves (Fig. 54) forming a cushion of a few inches in diameter, or its branches may be of some length, but bear terminally similar tufts of small leaves. Accompanying this rosette form of injury is a more or less yellowing of the foliage,
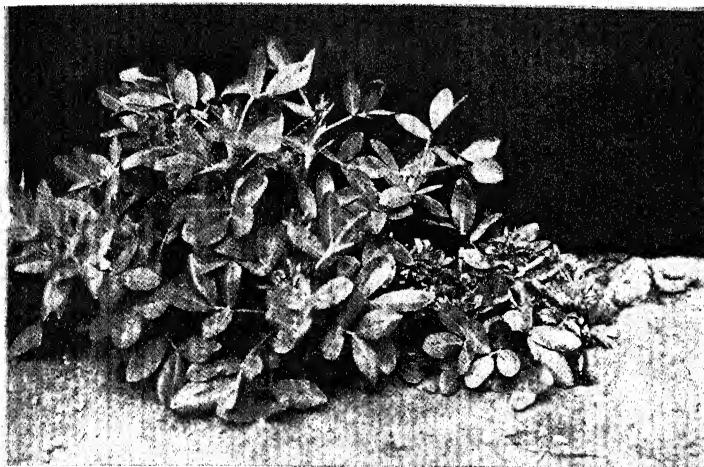
Fig. 54.—Rosette disease in Peanuts. Healthy and diseased peanut plants, the latter infected at an early stage of growth. (After Storey and Bottomley, *Anns. Appl. Biol.*)

usually distributed over each of the young leaves. Rosette disease up to the present has not been transmitted by inoculation of diseased sap, but in one instance grafting was found to be successful. Insect transmission with *A. leguminosæ*, however, proved highly successful in a large percentage of cases.

One of the most interesting cases of the relation of a virus disease to its insect carrier is that of the " aster yellows " disease which attacks the China aster, *Callistephus chinensis*, and is a serious disease in the United States.

Aster yellows is an infectious chlorosis, and its symptoms are

somewhat similar to the leaf-roll of potato, curly-top of sugar-beet and peach yellows. Diseased plants never show mottling, and it is therefore not a mosaic disease. The first symptom to be observed on the young plant is a slight yellowing along the veins in the whole or part of a young leaf. After a plant has been diseased for some time the new leaves that develop are chlorotic throughout. One-half or more, or a sector amounting to less than one-half of a plant, may be chlorotic for some time before the remaining portion is affected. Such sectorial infections are characteristic of aster yellows. The disease is systematic in the above ground portions of the plant and causes chlorosis in all green tissues. Curiously enough, however, petals which normally contain no chlorophyll become quite green in diseased plants. Another striking symptom of the disease is the abnormal production of secondary shoots. Instead of the lateral buds remaining dormant, they produce long, thin, chlorotic branches. Stunting of the plant, as well as of the flower heads, is also a feature of the disease. A peculiar characteristic of aster yellows disease is the change which it brings about in the response of the plants to gravity. Instead of the diseased leaves lying flat and making a broad angle with the perpendicular, they stand upright.

The disease can be transmitted to more than fifty species in twenty-three different families of plants, such as the Compositæ, Plantaginaceæ, Solanaceæ, Labiatæ, Cruciferæ, Chenopodiaceæ, to mention but a few. Among asters, however, the disease is only spread by the leafhopper, *Cicadula sexnotata*, and by no other insect. The relationship between virus and insect vector here is apparently of a highly specific nature.

It has been shown by Kunkel (1926) that the transmission of aster yellows by *Cicadula sexnotata* is not a simple mechanical transfer. In the first place, there is a definite period between the time virus-free insects feed on diseased plants and the time when they are able to transmit the disease—the incubation with... and secondly, the persistency of the virus in the insect. variabl... ...cubation period varies somewhat for different colonies of ture the s... such colonies are unable to transmit the disease until was betwee... ...feeding on diseased plants. In general terms, the

The rosette disea...

incubation period is shorter for adults than for nymphs, but is never less than ten days, and may be as long as three weeks.

Kunkel considers, from this intimate and specific relationship which exists between the virus and its insect vector, that the causative agent of aster yellows is biological rather than chemical in nature, for it is difficult to conceive of any agent other than a living organism that would require an incubation period in its insect carrier, or would be retained by the insect over long periods of time in the absence of susceptible host plants. Like leaf-roll of potato, aster yellows cannot be transmitted by juice inoculation, but it has been produced by budding.

The examples of virus diseases and their insect vectors could be multiplied indefinitely, and it will on this account be more profitable to discuss certain recent work on the separation of some of the mosaic disease of the potato into two or more entities by the aid of a combination of needle inoculation and insect vectors.

It was shown by Kenneth Smith (1927) that the mosaic disease of potato is transmitted by *Myzus persicœ* and *Macrosiphon gei*, but he was unable to produce the symptoms of mosaic in a potato plant in the same year as that in which the inoculations were performed by means of aphids placed upon the haulms, and the disease only showed itself in the plants produced by the progeny of the experimental plant in the following year. If, however, the aphids be placed upon sprouting tubers it appears possible to produce current-year symptoms.

It was later found by Kenneth Smith (1929) that needle inoculation of a potato mosaic virus into tobacco gave rise to a ringspot disease in the latter plant. It was further discovered that progressive needle inoculation of this ringspot disease into successive tobacco plants resulted in a greatly increased virulence of the disease. This increase in virulence was such as to cause the death of very young tobacco plants in every case and to produce a severe necrosis in older seedlings. The behaviour of the older seedlings was fairly constant; the necrotic disease killed every portion of the plant with the exception of the young central shoot. After a period of two to three weeks, the plant commenced to
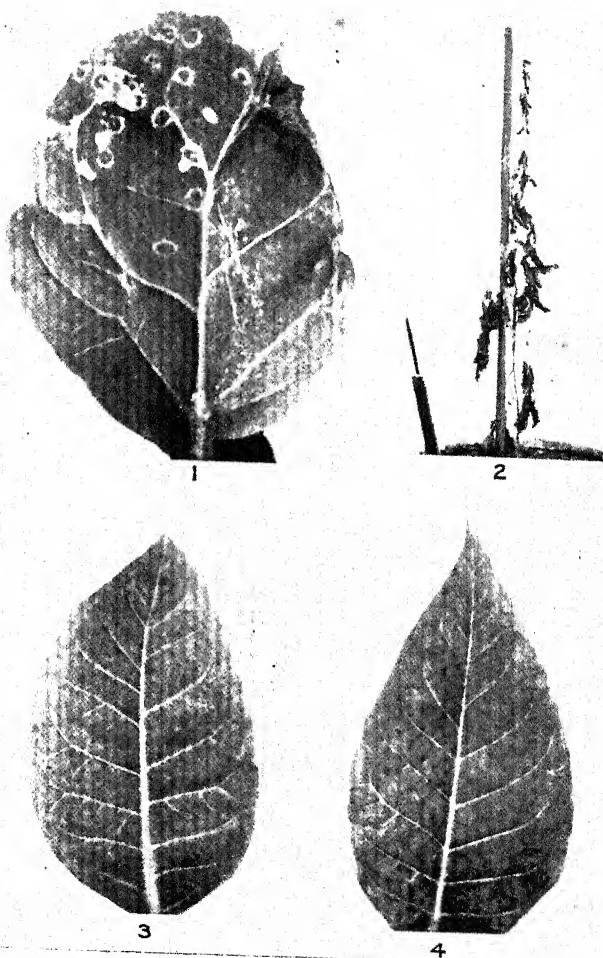
FIG. 55.—(1). Tobacco leaf (*Solanum tobacum*, var. Connecticut) infected by needle inoculation with potato mosaic, showing rings formed at point of inoculation. (Fourth successive inoculation. (2). Potato plant (*S. tuberosum*, var. Arran Victory), killed by needle inoculation with the virulent virus from tobacco. (3). Leaf of tobacco (White Burley), showing typical appearance of the aphis-induced disease. This leaf was from a plant which was right in a series of successive needle inoculations of the disease, after it had once been produced in tobacco by the aphis. No appreciable increase in virulence was obtained. (4). Leaf of White Burley tobacco affected with the aphis-induced disease. In this case, the disease after production in tobacco by the aphis, had been needle-inoculated through potato and back to tobacco. The symptoms remained unchanged. (After Kenneth Smith, *Anns. Appl. Biol.*)

grow once more, and the new leaves formed showed a severe and sometimes brilliant mosaic-like mottling.

When healthy potato plants are inoculated with the ordinary form of tobacco ringspot, a mosaic is developed in which the symptoms are intensified and the infective power greatly increased. But inoculation into the potato of the virulent form of tobacco ringspot, obtained in the manner described above, that is, ringspot passed through a successive series of tobacco plants, produces an additional factor. On the young leaves of a potato plant inoculated in this way there first appear the symptoms of an intensified mosaic, characteristic of ordinary ringspot inoculation; later, however, the lower leaves of the potato plant commence to die and death proceeds rapidly up the plant, the leaves first turning yellow and then shrivelling completely, and ultimately the whole plant may die (Fig. 55).

It was also found that aphids (*Myzus persicæ*) could be induced to carry infection with regularity to the healthy potato plant from tobacco affected with virulent ringspot. The symptoms produced on the potato were identical with those produced by aphis infection with ordinary tobacco ringspot, *i.e.*, an intensified form of mosaic. There is, however, this difference between aphis transmission and needle inoculation: if the disease be returned to tobacco from infected potato there is no decrease in the virulence of the disease in its passage through the potato, and tobacco seedlings rapidly die; but in the case of aphid transmission, if the disease be returned to tobacco by needle inoculation after it has passed through healthy potatoes, the tobacco shows the dark green lines typical of aphis infection. Comparison of the respective inoculations of healthy potato by aphis and needle from tobacco affected with the virulent virus, shows that the lethal character of the disease is not transmitted by the aphis.

Comparison of needle and aphis infection of tobacco with potato mosaic shows that needle inoculation produces ringspot on the tobacco, whereas the aphis produces a mottling accompanied by lines and spots of darker green, and the accompanying chart shows a diagrammatic representation of the resulting symptoms in each case (Fig. 56).

In the case of infection with aphids there was little or no increase in the virulence of the disease through successive inoculations, and it would appear that the aphid fails to transmit some element of the virus which is concerned with the production of
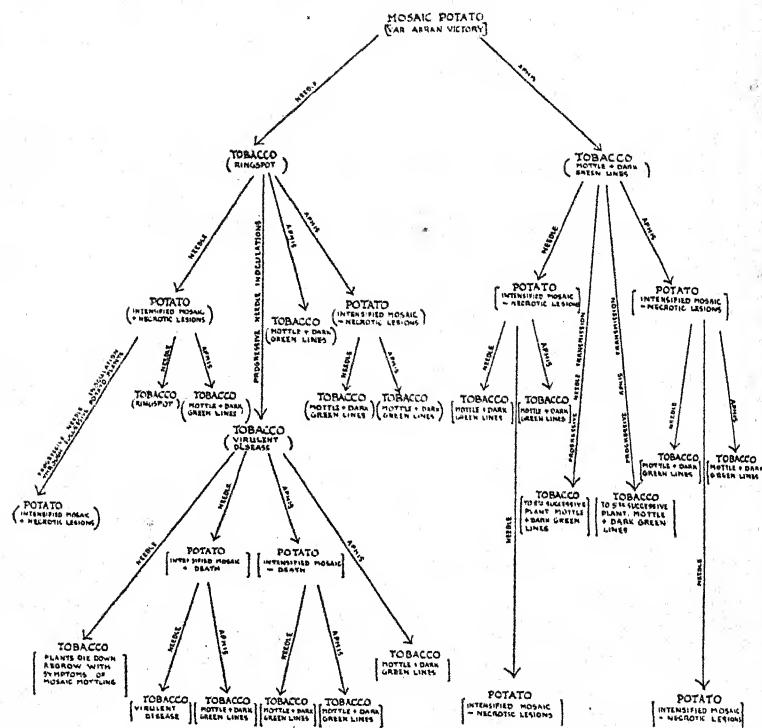
FIG. 56.

the more lethal and necrotic symptoms, which are carried over by needle inoculation.

Kenneth Smith (1931c) has now been able to show that certain potato viruses of the mosaic group are not single entities but are in reality made up of two or more complexes. The disease which exhibited itself with needle scratch on tobacco is a mixture of two complexes. In other words, needle inoculation passes over both diseases, which for the sake of clarity are called x and y ; x exhibits

itself on tobacco as ringspot, and y represents the aphis-borne virus, the symptoms of which take the form of a darkening of the dark green colour of the tissues along the veins in tobacco.

The methods of virus separation used in this investigation fall into three groups and separation was effected in the following ways :—

(i.) By utilising a selective relationship which exists between the aphis vector, *Myzus persicæ*, and the tobacco plant.

(ii.) By the use of " filter " plants. A comparative study of the host range of the single virus (y), isolated by means of the insect, and of the virus complex ($x + y$), revealed the fact that there were several plants which were susceptible to ringspot virus (x) but were resistant to the aphis-transmitted virus (y). This fact suggested the possibility of using certain of these plants as filters, and experiment has shown that passage of the whole complex ($x + y$) through such plants removes the aphis-borne virus (y). That the virus y is actually removed by this process was shown by the analysis and synthesis of the complex, and also by the fact that the aphis cannot pick up y from a ringspot resulting from the passage through one of the filter plants. The successful use of filter plants depends on needle inoculation, since grafting transmits the whole complex.

(iii.) By taking advantage of the unequal rates of movement of the constituent viruses within a plant host : (1) at the moment of development of primary symptoms in the young plant inoculated with the complex, and (2) in the ageing plant.

· Carriers

The presence of carriers of virus diseases in plants has been known for a considerable time, although actual details are rather meagre. For example, it was shown by Allard (1914) that tobacco mosaic could be transmitted to a number of species of *Nicotiana*, other than *N. tabacum*, but when juice was inoculated into *N. glutinosa* there was no appearance of symptoms. Nevertheless, the plant was carrying the disease, because when juice from apparently healthy *N. glutinosa*, which showed no signs of mosaic after inoculation, was returned to *N. tabacum*, the latter developed

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the disease. Walker (1925) and Elmer, however, have shown that *N. glutinosa* is not really immune and is a species susceptible to tobacco mosaic, although Allard failed to produce infection. Similarly, it has been shown by Kenneth Smith that the common solanaceous weed of cultivated and waste land, *Solanum nigrum*, is a carrier of potato mosaic. It is, however, doubtful whether *S. nigrum* plays any part in the over-wintering of potato mosaic, since it is an annual, and there is at present no evidence of seed transmission in this plant. Quanjer found that when he grafted to a healthy tomato plant the top of a leaf-roll potato, the tomato showed no signs of the disease, but if the potato graft were removed and a part of the apparently healthy tomato was in turn grafted on a healthy potato, the latter developed leaf-roll.

The curious results obtained by Johnson (1925) when he inoculated juice from apparently healthy potatoes into tobacco and found that the latter developed various symptoms are probably to be accounted for by the fact that his potato varieties were carriers.

Johnson found that when he inoculated the juice obtained from apparently normal plants of the variety Triumph into tobacco, three characteristically different symptoms were developed on the latter : (i.) a " mottle," (ii.) " spot-necrosis," and (iii.) " ringspot."

While not actually disregarding the fact that Triumph may be a carrier and is tolerant of certain infectious viruses, Johnson inclined to the view " that the viruses secured from healthy potatoes are not actually present in the potato as a true virus, but merely as normal (or possibly abnormal) protoplasm . . . a hypothesis possessing many advantages from the standpoint of explaining what is now known about virus diseases." Such an hypothesis, however, is no longer tenable. It is now well established that a number of potato varieties are carriers of virus diseases. For example, many plants of Up-to-Date carry a streak, while it has been shown by Salaman that all tubers of King Edward carry a disease termed by him " para-crinkle."

Both Henderson Smith (1928) and Salaman have independently failed to confirm Johnson's statements. Henderson Smith inoculated into the tomato, juice from nine different varieties of potato : Arran Victory, Arran Chief, Epicure, Sharpe's Express,

Majestic, Great Scot, President, Abundance, and King Edward, which had been carefully tested beforehand for the presence of any concealed virus that might be harboured, and in no case found any symptoms of virus disease were developed on the tomato. On the other hand, when potato mosaic was inoculated into tomato from diseased plants of Kerr's Pink, characteristic signs of disease were developed within fourteen days.

It seems clear from this work with the potato varieties that were used, that in this country potato protoplasm, as such, does not produce virus disease in tomato.

A particularly interesting case of a carrier of virus disease in the potato has been investigated by Salaman and Le Pelley. It was found by these investigators that when scions of apparently healthy King Edward were grafted on to Arran Victory the latter developed a severe " crinkle." When a similar operation was repeated using President in place of Arran Victory, the President showed no signs of disease, but nevertheless it was also acting as a carrier, since when Arran Victory was grafted on to the apparently healthy President, the former developed the same symptoms as when grafted alone to King Edward (Fig. 57).

The variety King Edward is an example of a really perfect carrier. The plants used by Salaman and Le Pelley were especially selected for their appearance of health. This latent disease, which is present in King Edward tubers, is termed by these authors " para-crinkle," to distinguish it from the crinkle found in other potato varieties to which Salaman has given the name of " crinkle A."

The clinical symptoms of para-crinkle on Arran Victory develop very irregularly. On occasion the disease may sweep over the whole plant in the course of twenty-four to forty-eight hours, and every leaf of the upper and younger parts of the plant is involved. When this happens, the first symptom is a very definite blotch with yellow spots, followed rapidly with deformity and waving of the leaf edges. The disease does not always develop with rapidity and when the symptoms appear more slowly, the sign is a single bright blotchy patch of chlorosis at the side of the larger veins, but even at the earliest stage this

FIG. 57.—(1). King Edward potato plant, healthy to all appearance, but carrying Para-crinkle. (2). An Arran Victory plant grafted with a President scion carrying Para-crinkle. The scion, which has grown vigorously, is perfectly healthy in appearance; the Arran Victory stock is showing severe crinkle. (3). A plant of Arran Victory raised from a tuber which had been infected two years previously with Para-crinkle. (4). An Arran Victory plant grown in the field raised from the tuber of a plant infected the previous season. The plant has developed " curly dwarf." (Salaman and Le Pelley, *Proc. Roy. Soc. (Lond.)*.)

panied by local puckering and deformity, allied with an early waving of the leaf edge. In the first season of the disease the symptoms are chiefly to be found in the younger upper growth, but in the second season of growth the stems are numerous, short and brittle, the foliage is clumped and grossly deformed, and all lesions are intensified. When grown in the field, the plants show a dwarfed appearance and are often not more than 6 or 9 inches high, the leaves are clumped and twisted and the plants look more like cabbages than potatoes.

The reactions of para-crinkle in other varieties than Arran Victory were also studied. In Arran Chief the same clinical picture was produced as in Arran Victory; in Arran Comrade, Great Scot and Majestic a mosaic was produced instead of a crinkle, while varieties which did not visibly react at all, but nevertheless carried the disease, were: Abundance, President, and possibly Champion, and lastly, the following varieties appeared to neither react to the virus nor did they appear to act as carriers: Arran Crest, Di-Vernon, Epicure and Sharpe's Express.

Unlike crinkle A, para-crinkle does not appear to be communicated to *Datura* by juice inoculation either directly from King Edward or indirectly from it through Arran Victory, nor is it transmitted by insects, but the disease can be transmitted to *Datura* by grafting. It was also found that double grafts made by grafting *Datura* stems (with and also without leaves attached) to Arran Victory and a para-crinkle scion to the *Datura*, that passage through the tissue of a solid, leafless *Datura* stem, 2 inches long, had no effect on the virus, but if the leaves of *Datura* were present, then the virus was destroyed by its passage through the *Datura* stem.

Ætiology

From time to time statements have been made, and descriptions published, of different organisms associated with one or more virus diseases of plants. For example, bacteria, protozoa and mycetozoa have been described as being present in plants infected with virus. Such an account was given in 1903 by Iwanowski in his investigations of tobacco mosaic. He discovered that cer

of the cells in diseased leaves contained plastic amœboid bodies, and that these were never found in tissues of healthy plants. He concluded, however, that since the entity which causes tobacco mosaic is capable of passing through the pores of a porcelain filter, the amœboid bodies must be the result rather than the cause of the disease. Similarly, Eckerson has described certain motile organisms in the leaves of tomato affected with mosaic, as well as in other plants suffering from virus diseases, *e.g.*, wheat rosette, mosaic in *Hippeastrum Johnsoni* and dahlia.

The fact that filters of a sufficient grade of fineness will retain plant viruses has led to the general belief that the causal agents of these diseases must be particulate, and the majority of investigators incline to the view that they are living parasites, although the evidence for this view is by no means conclusive. From filtration experiments, Duggar and Karrer place the size of the virus of tobacco mosaic at $30\mu\mu$. On the other hand, the various organisms that have been stated to be associated with these diseases are all of comparatively large size; but this is not a conclusive argument against their acceptance, since a part of their life-cycle may include an ultra-microscopic, or filtrable stage. However, the evidence of actual relationship between disease and the presence of these organisms is by no means satisfactory.

Olitsky stated that he was able to obtain infection with the twelfth sub-culture in sterilised juice of tomato mosaic and that there was multiplication *in vitro* of this virus. This statement, however, has never been substantiated by other workers. Henderson Smith (1927), Mulvania, Purdy and Goldsworthy all failed to find evidence for such multiplication, and at the present time no plant virus has been proved to multiply outside a living host.

In 1910 Lyon described the presence of small, spherical, irregularly shaped bodies in a disease of sugar-cane to which he gave the name of Fiji disease, as at that time it was very prevalent in Fiji. This Fiji disease of sugar-cane is an infectious gall disease, and elongated galls are produced at irregular intervals in the phlœm of both leaves and stalks of the plants. These bodies were believed by Lyon to be a parasitic organism. Some years later (1924) Kunkel discovered the presence of intracellular bodies in

the cells of chlorotic tissue of corn plants suffering from mosaic. In healthy plants such bodies were never present. He was also able to find such bodies associated with the mosaic disease of *Hippeastrum equestre*, and in both corn mosaic and in *H. equestre* these bodies were closely associated with the nucleus of the cell.

Since these earlier publications, such intracellular inclusions have been shown to be present in a considerable number of virus diseases of plants and in very diversified families. These bodies are practically always extranuclear, although Goldstein (1927) had recorded a single instance of an intranuclear inclusion in dahlia mosaic. Their production is dependent on the nature of the virus rather than on the nature of the host plant. In the case of tobacco mosaic these bodies are produced in every host in which the disease exhibits symptoms, whereas cucumber mosaic never forms them. The presence of such abnormal inclusions is not confined to plant viruses alone; they are to be found in the virus diseases of man, and the virus diseases of insects and fishes, and their appearance is so regular as to make them a valuable aid in diagnosis in certain instances, such as rabies.

These inclusions, which have been aptly named X-bodies by Goldstein, have been carefully studied in a number of instances, and a good deal of information is now available with regard to their behaviour and formation. Goldstein has made a particularly thorough investigation of X-bodies in living tissues of both growing-points and leaves of plants affected with tobacco mosaic. She was able to bring forward evidence that they divide by constriction and that their distribution in the plant is brought about by division of the host cells. More convincing evidence that X-bodies divided and were distributed by successive divisions of the host cells, was found by Goldstein in the mosaic disease of dahlias.

X-bodies are granular or finely reticulate in structure, and generally stain more or less deeply with aniline dyes. They are usually well defined, appear on occasion to have a bounding wall or membrane, and are rounded in shape. Though often roughly spherical, they are also frequently elongated, and are vacuolate, sometimes with only one very large vacuole. As a rule,

the margins of the vacuole stain more deeply than the rest of the mass. There is a considerable amount of evidence available that they are able to increase in size. In the early stages of the disease they are small, but become larger in the more advanced stages. There is no direct evidence of autonomous movement, but the bodies move freely about the cell in the streaming of the protoplasm.

It has been established by Holmes that in the mosaic disease of *Hippeastrum equestre* mitochondria are present in the X-bodies. These mitochondria are well distributed through the substance of the X-bodies, and Holmes inclined to the view that the intracellular body in this particular disease consists of living cytoplasm, but against this view must be set the fact that he was quite unable to discover the presence of a nucleus. (Fig. 58.)

A number of workers have tended towards the view that X-bodies are living entities, and the evidence upon which this conclusion is based is the fact that (*a*) the X-body looks like an amœba in general appearance, (*b*) pseudopodium-like projections are sometimes present which suggests amœboid movement, (*c*) the appearance of fission and occurrence of several bodies in the same cell which suggests that division has taken place, (*d*) increase in size, and (*e*) their protein nature and the presence of mitochondria. The recent observations of Sheffield and Henderson Smith on their formation and nature, from their first appearance in the hairs of *Solanum nodiflorum*, when infected with aucuba mosaic of tomato, make it unlikely that they are actually living entities.

Solanum nodiflorum is a particularly suitable subject for an investigation of this nature. The X-bodies are conspicuous structures in the cells of the leaf-hairs in this plant, and they occur unaccompanied by those other abnormal inclusions (such as striate material, crystal plates, etc.) which crowd the cells in many hosts and complicate the issue. Aucuba mosaic of tomato causes a typical mosaic in *S. nodiflorum*, with characteristic irregular mottling of the leaves, and is transmitted to this species with great ease by juice inoculation.

The hairs on the leaf are two- or four-celled structures, with somewhat rigid walls, and they stand out stiffly from the epidermis.
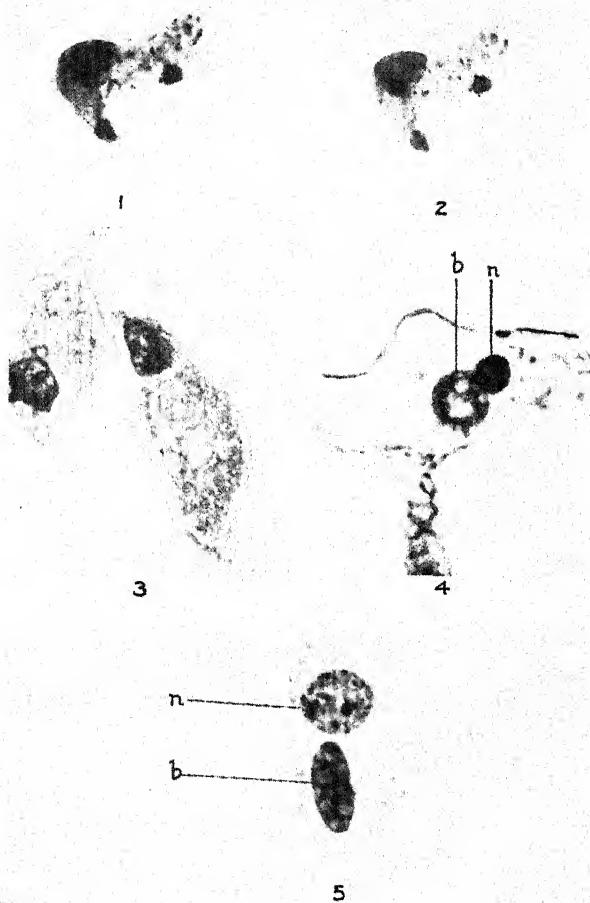
FIG. 58.—Examples of X-bodies. *Hippeastrum* mosaic. (1) and
(2). Nucleus and adjacent intracellular body, photographed
at different optical levels to show the distribution of dot-like
chondriosomes through the mass of the body, and not merely at
the surface. (3). Intracellular body, showing granular structure.
(4) X-body from a palisade cell of a potato leaf (var. Green
Mountain) suffering from crinkle. X-body (*b*), nucleus (*n*).
(5). Tobacco mosaic on tobacco. X-body in a hair-cell.
X-body (*b*), nucleus,(*n*). ((1), (2) and (3), after Holmes,
Bot. Gaz. (4) and (5), after Hoggan, *J. Agric. Res.*)

The cells of the normal hair contain nothing but the nucleus, the peripheral cytoplasm, and a varying number of strands of streaming cytoplasm. No chloroplasts are present. The hairs of an infected leaf contain, in addition, a large abnormal inclusion, the X-body. Typically, these X-bodies are roughly spherical, and usually there is a tendency for the rounding of the contours. The size varies very materially; the larger may be as much as 30μ in diameter, while the smaller may be of the order of 5μ or less in their larger diameter. The larger bodies are partially translucent and are tinted brown or pale yellow, while the smaller bodies may show no colour. In structure they are coarsely granular, and in many cases look as if they were aggregations of smaller particles rather than being truly homogeneous. Vacuoles are present, and the number varies from as many as nine to one or two. In most cases the body is found in close contact with the nucleus, sometimes alongside, often more or less enveloping it, but never incorporated with it.

Fig. 59. — *Solanum nodiflorum*. Hair showing X-bodies. Note the granular appearance; and in the two terminal cells the projecting particles. In the third cell the nucleus is visible at the upper edge of the body. (After Henderson Smith, *Anns. Appl. Biol.*)

In *Solanum nodiflorum* there is a pronounced tendency to crystallisation in these bodies, a fact not previously recorded in the literature. This is very clearly to be seen on the surface of the larger X-bodies, in which definite crystals can be seen to project from the mass, and are particularly visible at the margins, but they are usually best observed in the elongated forms lying along the walls of the cell. This tendency to crystallisation is accentuated in certain fixatives, such as Bouin and Carnoy, but it can also be seen
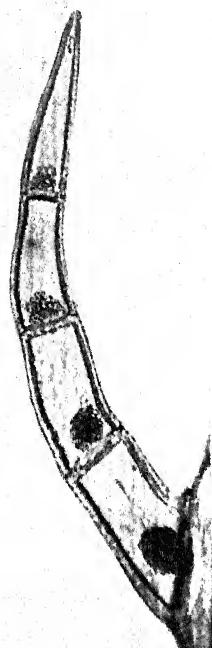
in unfixed material. It is most evident in old leaves and in leaves in which infection has been of long duration.

In the early stages of infection there appear in the streaming cytoplasm of the hair small particles, which are carried along in the stream and tend to aggregate together in the formation of larger masses. With increase in time, these particles extend in size, and at the anastomoses of the strands, tend to hesitate, or halt, for a longer or shorter time before they move forward once
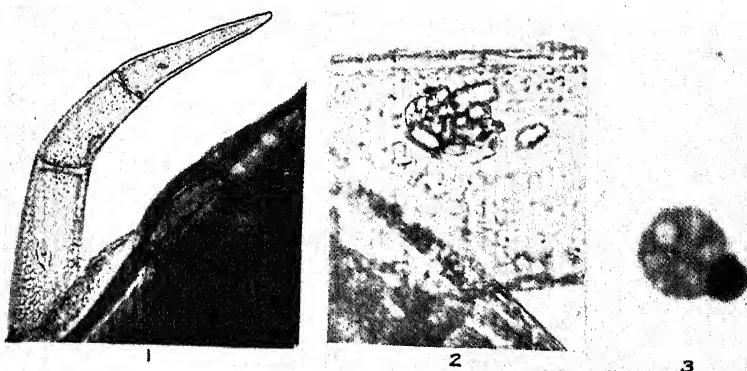
FIG. 60.—*Solanum nodiflorum.* (1). A normal hair. (2). Crystallising X-body in leaf-hair. The nucleus can be partly seen at left upper edge of the mass of crystals. (3). X-body in epidermis of leaf. The smaller dark body is the nucleus. (After Henderson Smith, *Anns. Appl. Biol.*)

more, their further progress being facilitated by modifications and alterations in shape of the plastic particles themselves. With still further increase in size, the halts become longer in duration, and they may last for a couple of hours or more. At such a halt another particle may join the stationary one, and when the movement is resumed, the two may separate, or they may go forward as one mass. By successive increments large masses are eventually formed which are readily recognisable as X-bodies. These composite masses may remain permanently in union, and when they do, they appear to fuse together into a more homogeneous whole, in which vacuolation may be observed. Occa-

sionally, however, a composite mass may separate once more, even after long contact, into its constituent parts, which resume their independent movement. When this takes place, figures are seen which show every appearance of fission, but this is not a case of division in the sense of multiplication, since the separate bodies may once more unite. Similarly, when a smaller mass breaks away from, or unites with, a larger mass, appearances are presented which simulate the production of pseudopodia. This mode of formation accounts for many of the appearances which have led to the belief that X-bodies are living entities. It also explains the great tendency for X-bodies to be associated with the nucleus, since it is round the nucleus that the protoplasmic strands cross and anastomose most freely, and such situations allow of aggregations to take place.

A number of chemical tests were made with these X-bodies in an attempt to elucidate their nature. They are able to withstand boiling in distilled water for twenty minutes, and are not dissolved in alcohol of any strength, nor are they soluble in acetone or chloroform. They dissolve in either caustic soda or potash, and they are also soluble in hydrochloric and sulphuric acid. With Millon's reagent they give a pronounced brick-red colour, and they also give the Biuret reaction. Potassium was also found to be present, but they gave no reactions for fats with Sudan III, Sharlach R, or osmic acid.

It is clear from these various reactions that the X-bodies in *Solanum nodiflorum* are protein in nature, and are regarded by Henderson Smith (1930) as a product of the reaction produced in the cell cytoplasm by the virus and not as any living organism, and certainly the evidence produced of the method of formation and the chemical reactions of these bodies seems to point to the fact that this conclusion is correct.

Physiology of Virus Diseases in Plants

Up to the present the physiological side of the virus diseases in plants has been scarcely touched upon, and such papers as have been produced are all of a very poor standard of quality. The majority of investigators appear to have studiously neglected this

aspect of the subject, and to have concentrated on inoculating expressed juice into any number of host plants and observing the appearance of symptoms, making thereby an already confused subject worse confounded by recording each fresh symptom as a new disease with a new name. If the literature of the virus diseases of plants be reviewed as a whole, it must be confessed that up to now the greatest progress has been made on the entomological side in relation to the transmission of these diseases by their insect vectors, and the purely botanical side, physiological and otherwise, makes but a sorry picture in comparison.

An attempt has been made by the Dutch investigators under Dr. Quanjer at Wageningen to elucidate the physiology of potato leaf-roll. Two prominent features of leaf-roll in potatoes are: (i.) accumulation of starch in the lamina of the leaf, and (ii.) necrosis of the phlœm. Three main hypotheses have been put forward to account for the accumulation of starch in the blades of the leaves: Oortwijn Botjes, Woods, and others, consider that it is due to a disturbed enzymic process, while Quanjer holds that it is due to disturbed transport on account of the necrosis of the phlœm, which is the main downward channel for carbohydrates. Murphy (1923), on the other hand, put forward the suggestion that transport does not take place because the plastic materials are not present in a water-soluble condition. Thung has made an investigation of this problem to see which of these hypotheses is correct.

The potato variety used was Paul Kruger (probably identical with our variety, President). Secondary leaf-roll was obtained by planting diseased tubers, while the disease in the primary condition was obtained by infection with *Myzus persicæ*. It might be suggested, in parenthesis, that since the plants were grown in a greenhouse, the leaf-roll obtained by insect transmission was also in the secondary stage, as the primary stage only exists for a very short time under greenhouse conditions.

The rate of photosynthesis in young leaves of diseased plants was found to be normal, but decreased with accumulation of starch. The respiration rate of diseased leaves appeared to be higher than that of normal, healthy leaves. In plants which had been darkened there was loss in dry weight in the case of healthy plants,

presumably due to transport of sugars from the blade as well as respiration, whereas in the leaves of the leaf-roll plants the loss in weight did not appreciably differ from the amount lost from respiration. It is concluded from this that there is no transport in the diseased plants and that accumulation of starch is due to necrosis of the normal path of transport, *i.e.*, the phlœm. There is one serious difficulty in accepting the theory of a disturbed path of transport, for it has been shown by Murphy that accumulation of starch takes place *before* and not after necrosis of the phlœm.

Some unpublished observations of the present author and his colleague, Mr. Alan McBain, bear on this problem of the accumulation of starch in potatoes affected with leaf-roll. In this investigation two varieties were used, Arran Victory and President. The disease was transmitted by sprout infection with *Myzus persicæ*, and no difficulty was encountered in inducing the disease, and 100 per cent. infection was obtained in both cases. The plants, diseased and healthy controls, were grown in insect-proof greenhouses, and in no case did the investigators observe more than the transient appearance of the primary symptoms of leaf-roll, and the plants passed quickly into the secondary condition.

It was decided to investigate the carbohydrate changes in laminæ and petioles at hourly intervals over twenty-four hours at different times in the growing season, and the experimental runs were arranged accordingly, two on President and three on Arran Victory. The carbohydrates estimated were, hexoses (glucose and fructose), sucrose and starch, and the results were calculated as a percentage of the " residual dry weight," *i.e.*, dry weight less total carbohydrates, which gives a more constant basis for calculation over short periods of time than the total dry weight, since the latter value will vary with the amounts of carbohydrate present. The hourly variations in temperature and radiation were also recorded.

The data were statistically examined by the methods given by Fisher and by " Student " and a few of the results and conclusions are given below.

In the earlier part of the growing season, end of May and early June, it was found that in the normal plants there was a rise in the

hexose values with rise in radiation, and increase in sucrose lagged behind increase in hexose. Over the whole experimental period there was a negative correlation with time in the hexose values ($r = -0.712$) and a positive correlation with time in the sucrose values ($r = +0.651$). The starch, like the hexose, showed a negative correlation with time, but the value was not statistically significant ($r = -0.440$). The direct correlations between hexose and sucrose and sucrose and starch were not statistically significant, but the direct correlation between hexose and starch was statistically significant ($r = 0.5430$). On the other hand, when the partial correlation between hexose and sucrose, with time kept constant, was calculated, the value between the two showed a high significant correlation ($r = 0.8327$). Similarly, there was a high significant partial correlation between sucrose and starch, with time constant ($r = 0.6387$). When the partial correlation between hexose and starch was calculated, with both sucrose and time kept constant, the value was still statistically significant, but negative in sign ($r = -0.8373$). The relationship of the sugars with temperature was complex. None of the direct correlations between either hexose, sucrose or starch with temperature were statistically significant, but when the partial correlations were calculated, with time and one of the sugars kept constant, then it was ascertained that the partial correlation between hexose and temperature, with sucrose and time kept constant, became statistically significant ($r = 0.7742$), while the partial correlation between sucrose and temperature, with time and hexose constant, was also statistically significant, but negative in sign ($r = -0.5704$), and the partial correlation between sucrose and temperature with time and starch eliminated was still statistically significant and negative in sign, but showed an increase over the partial correlation between sucrose and temperature with time and hexose kept constant ($r = -0.8950$ compared with $r = -0.5704$). In other words, the higher the temperature the more hexose is formed, while lower temperature favours the production of sucrose. Neither direct nor partial correlations between starch and temperature showed any significant correlations.

The fact that hexose rises with radiation and sucrose lags

behind, and the further fact that there is a negative correlation between hexose and time, and a positive correlation between sucrose and time, as well as the fact that temperature and hexose show a positive value, whereas sucrose shows a negative correlation, points almost irresistibly to the conclusion that hexose, and not sucrose, is the first sugar of photosynthesis in normal potatoes.

In normal potatoes, statistical examination showed that there was a correlation between sucrose in the lamina and sucrose in the petiole ($r = 0.5431$), and also between the total sugars (hexose and sucrose) in the lamina and sucrose in the petiole ($r = 0.5862$) so that the translocatory carbohydrate in the healthy potato is sucrose.

Plants affected with leaf-roll also exhibited an increase of hexose with radiation, but the values lagged behind those of the healthy controls, and on the average there was lag of about two hours between increase of hexose in the healthy and increase of hexose in the leaf-roll plants. The variation with time in the hexose values was again negative, but the value was not statistically significant ($r = - 0.044$). The behaviour of sucrose, however, was peculiar. There was a slow rise in the sucrose values during the day, and then, with remarkable suddenness, they surged to a maximum and the correlation between sucrose and time was positive, and statistically significant ($r = + 0.8010$). The variation of starch with time was found to be positive but not statistically significant ($r = + 0.061$). All possible correlations between hexose, sucrose and starch were calculated, but the only significant correlation that was obtained was between sucrose and starch, in which the partial correlation with time eliminated was ($r = 0.5440$) and with time and hexose eliminated ($r = 0.5431$).

Analysis revealed the fact that in the petioles of the leaf-roll plants, *no* sucrose was present at any time of the day or night, and the only carbohydrates that could be discovered were hexose and starch, and that there was a direct correlation between them in the petioles ($r = 0.7200$). No correlations that were statistically significant could be discovered between the laminæ carbohydrates and the petiole carbohydrates. It should also be mentioned that, as far as could be ascertained from micro-chemical tests, the whole

of the starch in the petiole was to be located in the ground parenchyma.

It is not possible at this stage to give more than a few tentative conclusions from the facts that have thus far been examined. It seems clear that the disease in some way slows down the formation of sugars in the leaf during photosynthesis, if the hypothesis be accepted that hexose is the first sugar of photosynthesis. The rapid rise in the sucrose curve towards the end of the day is very characteristic, and occurred in every case in which the laminæ of the diseased plants were examined. Since sucrose is the sugar of translocation in the normal potato, it would appear that this sudden increase to a maximum in the diseased plants is due to the fact that sucrose is unable to escape from the laminæ.

Translocation, however, is the most difficult problem to explain in the leaf-roll plants. That translocation in some form must take place is evident from the fact that tubers are produced in the diseased plants. It will be recalled that in the petioles of leaf-roll plants no sucrose is present at all. Moreover, in leaf-roll plants the phloem which forms the downward channel for transport of sugars suffers necrosis, so that the normal path of translocation is barred. It will also be remembered that the starch in the petioles was located in the ground parenchyma, and that there was a direct correlation between hexose and starch in the petiole. It is therefore suggested now, that translocation takes place in the leaf-roll plants by a slow leakage downwards to the tubers of hexose *via* the ground parenchyma. If this hypothesis be correct, it may also account to a certain extent for the poorness in yield and size of tubers from leaf-roll plants, since the sugar that travels down to the tubers is not the normal one (sucrose) and hexose may not be so suitable for tuber formation.

The results obtained towards the end of the growing season, end of July and beginning of August, differed somewhat from those recorded above, inasmuch as the starch in the normal plants showed a positive and statistically significant variation with time, whereas the sucrose showed a negative variation with time, but the value was not statistically significant. In the leaf-roll plants there was still a positive and statistically significant variation in the sucrose

values with time, as in the early part of the growing season. Until the whole of the data have been examined, these tentative conclusions must remain.

According to Bewley and Bolas, if the expressed juice from a tomato plant infected with aucuba mosaic be mixed with an aqueous, colloidal solution of tomato chlorophyll, that is, the mixed green pigments extracted by the method of Willstätter and Stoll, from a healthy tomato plant, a marked reaction occurs under suitable conditions. The most striking manifestation of this reaction is the formation of a brown colour, and apparently the destruction of a greater or lesser amount of the chlorophyll. This browning action was found to be very much less intense in the sap from healthy plants. Light appears to have a very stimulating effect upon the reaction, and with a light intensity of 800 foot candles, at a temperature of about 30° C., the reaction was greatly accelerated. This curious reaction was apparently not correlated with the oxidase or peroxidase content of the sap, nor did it occur to the same degree with a sample of infected sap which had been boiled for a few seconds. If this reaction can be confirmed, and the conditions for its formation satisfactorily standardised, though this has not been done up to the present time, it might form a quick and quantitative method of studying the nature of a virus and its reactions *in vitro*.

Bolas and Bewley claim that the following points obtain in the metabolism of tomato plants affected with aucuba mosaic. In the early stages of infection the removal of starch from the leaves of plants placed in darkness is greatly accelerated, except at the points of infection, which show a marked local inhibition of starch removal. At this stage starch formation does not appear to be affected by light. This local inhibition of starch at the points of infection is, at a later stage, about fourteen days, followed by removal of starch over a larger area of the inoculated leaf, together with a slight yellowing of the chlorophyll and a failure to form starch over this portion in the light.

The acidity of an aqueous extract of infected leaves, which were sampled at dawn, *i.e.*, immediately after loss of starch, was found to be greater than from healthy leaves, and the local absence of

starch in the leaves appeared to precede the appearance of mosaic symptoms. At a still later stage of infection, some days or weeks after typical mottling has appeared, a marked accumulation of starch was found in parts of the infected leaf and complete absence in other parts. The following scheme is suggested for the starch metabolism of tomato plants suffering from aucuba mosaic.

$$\text{Starch}$$
$$\downarrow$$
$$\text{Action of virus}$$
$$\downarrow$$
$$\text{Acids}$$

Plus Nitrogen	Attack on chlorophyll	Respiration
$\downarrow$	$\downarrow$	$\downarrow$
Protein	Mottling, etc.	CO_2
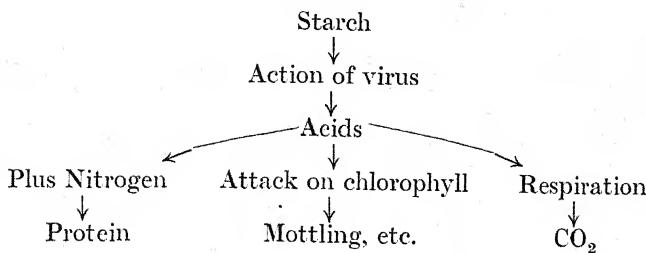
A number of investigations have been made in India on the physiology of the spike disease of sandal (*Santalum album*). In spike disease, as in leaf-roll, there is accumulation of starch in the laminæ of the leaves. It was found by Iyengar, as a result of a physico-chemical examination of the leaf-sap of normal and diseased leaves, that the hydrogen-ion concentration (pH) in the latter is lower than in normal, healthy leaves. In healthy leaves the pH of the sap lies between 5·15 and 5·71, whereas in the diseased leaves the range lies between 4·69 and 4·99. The results of a chemical analysis showed that the diseased leaves contained more free reducing sugars, starch, total nitrogen, and dry matter than healthy leaves. It was also ascertained that the moisture content was lower in diseased leaves, and they were also found to be deficient in potassium and calcium.

The diastatic activity of spiked and healthy leaves has been examined by Sreenivasaya and Sastri, who ascertained that the diastatic activity of diseased foliage is greater than healthy, the values of the former often being as much as four times as large as in normal foliage. One marked feature of the disease is apparently deficiency of calcium in diseased as compared with healthy leaves. Neither Iyengar nor Sreenivasaya and Sastri, however, attempt any theoretical deductions from their work.

The movement of the causative agent of aucuba mosaic in tomato has been investigated by Caldwell. The movement of virus in the host has been spasmodically worked upon from time to time. For example, it was shown by Bennett that the virus of " raspberry curl " could be confined indefinitely to the inoculated shoot by " ringing." If a small bridge of bark were left, the passage of virus from the inoculated part to the portions below took place quite readily, and he likened the movement of virus in the plant from the root of the resting stool, to the movement of food, the virus moving presumably in the living tissues. The rate of movement has also been examined by different investigators. Severin concluded that the infective agent of " curly-top " of sugar-beet on an average moved 4 inches per hour in his experiments, while Storey (1928) found that in six cases out of sixteen the rate of movement of the causative agent of mosaic disease of maize was from 10 to 20 cm. per hour.

According to Caldwell (1930), aucuba mosaic only moves in living tissue. If a portion of the stem of a plant be killed either by steam or chloroform, and the portion above or below be inoculated with infected juice, then the virus is unable to pass across the dead gap of tissue and is confined to the part of the plant that has been inoculated, that is to say, if the portion above the dead region has been infected, then only the upper parts of the plant show the symptoms of the disease and *vice versâ*. Movement of the virus is apparently not confined to the vascular tissues, since inoculation of infected juice causes systematic infection in all treated plants, and there is no apparent localisation of movement such as would be expected if the virus were travelling in the vascular tissues alone.

In a later paper (1931) Caldwell claimed that the virus could be induced to travel in the xylem under certain particular conditions. It was found, for example, that filtered virus juice could be induced to enter at the cut end of a petiole and that it travelled from there into the xylem of the main stem, and unless the petiole were removed within forty-eight hours from the time of infection, the experimental plant developed symptoms of mosaic. It was further discovered that if, after the removal of the petiole, the

leaves above were crushed, infection resulted. The virus, in other words, had entered and was travelling in the xylem, but was unable to pass out and cause infection. When the leaves above were crushed, the xylem was broken and the infecting principal was able to escape and brought about the disease. Combination of this experiment with steaming a portion of the main stem and then crushing the leaves still caused infection to take place, so that it is evident that the virus was being mechanically carried along in the xylem stream, but there is no mechanism to allow of its leaving the vessels. It would therefore appear that infection can only be brought about through broken cells, and that once the virus has entered the plant it travels through the ground tissue.

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